BALANCING THE COSTS OF INBREEDING IN COOPERATIVE CROWS

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Andrea Townsend

August 2009

BALANCING THE COSTS OF INBREEDING IN COOPERATIVE CROWS

Andrea Townsend, Ph. D.

Cornell University 2009

Because the cooperatively breeding American crow (*Corvus brachrhynchos*) exhibits natal philopatry of both sexes, related adults of the opposite sex interact, increasing the probability of matings between kin. Cooperative breeding based on natal philopatry of both sexes might be expected to persist as a strategy if inbreeding is avoided altogether, if inbreeding occurs with low or no costs, or if the benefits of living and breeding with kin outweigh inbreeding costs. In a suburban population of crows in Ithaca, New York, I found that 19% of social pairs were related at the level of first- or second-order kin, and that kin matings had severe costs for inbred offspring in terms of reduced body condition and immunocompetence, higher disease probability, and lower survival probability. Ithaca crows were not genetically monogamous: I found that 17% of offspring were sired by extrapair males, and that a male was more likely to lose paternity when he was injured, potentially due, in part, to a reduction in his functional fertility. I found no evidence that extrapair males were less related to a female than she was to her social pair male, no evidence that prospective extrapair males were more successful when they were less related to a given female, and no evidence that there were any genetic benefits gained by females through extrapair paternity. Furthermore, some within-group extrapair sires were sons of the breeding female, and incestuously produced offspring appeared to suffer the most severe costs. On the level of overall brood output, however, these genetic costs appeared to be outweighed by the parental contributions provided by within-group extrapair sires:

overall provisioning rate and brood output was higher in broods associated with within-group extrapair auxiliary sires, suggesting that these extrapair sires provided direct benefits to all young from broods in which they shared paternity. Another potential reason for the persistence of natal philopatry in this suburban population, despite severe inbreeding costs, is that the costs of inbreeding are lower in adjacent, contiguous rural populations, dampening an evolutionary response to selection against inbreeding.

BIOGRAPHICAL SKETCH

Andrea Townsend was born in 1976 in the suburban town of Burlington, Massachusetts, best known for its mall and for its fast highway access to Boston. Although these were dubious beginnings for an ecologist, she was fortunate enough that bordering her backyard was a small tract of trashy, polluted wetland that managed to elude development. This tract of forest was home to a struggling population of birds, rats, moles, snakes, and other wildlife. She spent her formative years watching and tracking these animals, earning her the nickname of "swamp-thing" among her understanding and supportive peers. She was delighted to discover, early on, that her proclivities for watching animals (combined with her strong interest in conserving them) could be turned into a career. During and after her undergraduate education in Biology/Environmental Studies at Bowdoin College, she assisted in many projects that had the goal of assessing the response of wildlife to habitat changes, such as the effects of different logging practices on breeding bird communities in Missouri, the condition of migratory birds during stop-overs in Costa Rica, the reliance of critically endangered Orange-bellied Parrots (*Neophema chrysogaster*) on human food sources in Tasmania, the use of Dippers (*Cinclus mexicanus*) as an indicator species for endangered salmonids, the interplay between urban wildlife and human Lyme disease risk, and the recovery of oaks after defoliation by exotic moths (*Lymantria dispar*). Although she was fascinated by each of these projects, Andrea became more and more excited about carrying out work of her own conception and design.

Andrea entered the graduate program at Cornell in 2002. She immediately commenced a comparative phylogeographic study on the Caribbean island of Hispaniola, focusing on endemic, high-elevation birds, many of which are severely threatened by rapid habitat loss. By mule, truck, small plane, motorcycle, and foot, in

collaboration with her husband/ fellow biologist, Jason Townsend, she conducted an island-wide survey of avian diversity. The papers that have been generated from this work emphasize the dire conservation status of evolutionarily distinct bird populations in Haiti.

In Hispaniola, Andrea was introduced to a phenomenon that she had never noticed before: cooperative breeding. She was filled with a passionate desire to understand it. In fact, cooperatively breeding birds excited her so much that she had no choice but to alter primary focus of her dissertation. To facilitate this drastic dissertation shift, she chose to work the only species of cooperatively breeding bird found on the Cornell campus: the American Crow *(Corvus brachrhynchos*). As luck would have it, these crows were part of a study population in which the birds had been marked and studied, demographically and behaviorally, for two decades. When she joined the Crow Research Group in 2005, the population was recovering from a recent West Nile virus epidemic. She found herself fascinated with the idea of how infectious diseases might affect the interactions among group members. Would group members help a sick family member to increase its probability of survival? Or would they evict a sick family member, in order to reduce their own infection risk? The chapters that follow begin to tell this story.

For Jason and Ava

ACKNOWLEDGMENTS

Many thanks to my advisor, Irby Lovette, for allowing me (and enabling me) to strike out in my own direction and follow my passions. Thanks to my other committee members- John Fitzpatrick, who inspired me through his enthusiasm and excellent advice; to Janis Dickinson, who was extremely generous with her time and energy in helping me become a better scientist; to Paul Sherman, who was the first to introduce and welcome me into the Department of Neurobiology and Behavior's lunch bunch, which greatly influenced my interests and thinking; and to Anne Clark, who welcomed me into the Crow Research Group with great kindness and enthusiasm, who has worked with me closely to ensure my dissertation work's success. This work could not have been conducted without the persistence, dedication, and climbing skill Kevin McGowan, who began marking the Ithaca crows in 1989. I also thank the undergraduate students that have assisted me with my research, particularly Laura Shoenle, who designed the crow microsatellite library for her honors thesis; Matt Savoca, who helped with behavioral observations; and Nick Sly, who has taken on much of the Hispaniolan phylogeography work for his honors thesis and beyond it. Thanks to Laura Stenzler, Chris Makarewich, and Amanda Talaba for the lab training and advice, as well as their patience in putting up with my mountain of samples, and to Elizabeth Buckles, Andrew Miller and Amy Glaser for their disease diagnostic work. Thanks to Doug Robinson, Walt Koenig, Evan Cooch, Kern Reeve, the Lovette lab and the Dickinson lab for advice and discussion. Thanks also to the Cornell Statistical Consulting Unit for its endlessly helpful advice. Support for this work was provided by the National Science Foundation, the National Institute of Health, the Animal Behaviour Society, Cornell Sigma Xi Grant-in-Aid of Research, the Frank M. Chapman Memorial Fund, the Kieckhefer Adirondacks Fellowship, the Cooper

Ornithological Society, the Wilson Ornithological Society, the Andrew W. Mellon Foundation, an Eloise Gerry Fellowship from Sigma Delta Epsilon/Graduate Women in Science, and the American Association of University Women.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE LOCI IN THE AMERICAN CROW*

---

*L. A. Schoenle, A. K. Townsend, and I. J. Lovette. 2007. Isolation and characterization of microsatellite loci in a cooperatively breeding corvid, the American crow (*Corvus brachyrhynchos*). Molecular Ecology Notes 7:46-48. Reprinted with permission from Wiley-Blackwell .

Abstract

I describe 11 microsatellite loci isolated from the American Crow (*Corvus brachyrhynchos*), the most wide-spread cooperatively-breeding bird in North America. Polymorphism at these loci ranged from 4 to 43 alleles, and observed heterozygosities from 0.53 to 0.92. Genetic parentage analyses using these markers will allow us to describe the mating system of this common but socially complex species, and to interpret their behavioral interactions in light of relatedness.

Introduction

Mating strategies vary extensively in cooperative breeders of the avian family Corvidae. One or several females may contribute eggs to the nest, and reported rates of extra-pair paternity range from 0-40% (Quinn et al. 1999; Li and Brown 2000). Females may copulate with extra-group breeders or auxiliaries (Williams 2004), within-group auxiliary males (Li and Brown 2000), or floaters (Berg 2005). Here, we design microsatellite markers for exploring the mating strategies of the American Crow (*Corvus brachyrhynchos*), the most wide-spread cooperative breeder in North America. Although the social behavior of crows has been studied intensively, the lack of markers has precluded the study of their genetic mating system.

Methods

American Crow DNA was isolated from whole blood collected in lysis buffer (Hoelzel 1992) using Perfect gDNA Blood Mini^{TM} Kits (Eppendorf, Hamburg, Germany). A library enriched for microsatellite sequences was created following the procedure described in Stenzler and Fitzpatrick (2002) with the following modifications. We digested genomic DNA in two separate reactions with the restriction enzymes *Alu*I and *Hae*III (New England BioLabs**)**, and incubated the

mixtures in their separate reaction tubes with the SNX linkers at 37º C for 30 minutes. After the addition of T4 DNA ligase, the reactions were left at room temperature overnight, and incubated at 80º C for 30 minutes prior to enrichment for repeat sequences. Biotinylated oligonucleotides were used to enrich the genomic DNA for 29 dimeric, trimeric, and tetrameric repeat sequences. Positive colonies were sampled and incubated in AE buffer at 97º C for 20 minutes to release the plasmid DNA from the *E. coli* cells.

We sequenced a total of 250 positive plasmid clones on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using BigDye Termination Mix (Applied Biosystems) with M13 forward and reverse primers (Integrated DNA Technologies). Sequences were analyzed and aligned using Sequencher 4.5 software (Gene Codes). Of the 250 clones, 64 (25.6%) contained repetitive elements. We developed primers for 35 sequences with suitable flanking regions, using the program PrimerSelect (DNA* Star). Ten of these loci exhibited poor amplification after preliminary PCR screening and were not tested further. We successfully optimized primers for the remaining 25 loci and tested them for variability on 15 individuals from a study population in Ithaca, NY. PCR reactions were performed in 48 and 24-well plates using a DYAD® thermal cycler (MJ Research). The cycling profile was one cycle at 94º C for one minute, 35 cycles of one minute at 94º C, one minute at the locus-specific annealing temperature (Table 1.1), and one minute at 72º C, followed by a final extension cycle of five minutes at 72º C. Reactions (10 μL) contained 10-100 ng of genomic DNA, 0.75 U of Jumpstart^{TM} *Taq* Polymerase (Sigma, St. Louis, MO), 10 mM Tris-HCL (pH 8.3), 50 mM KCl, $MgCl_2$ specific to each locus (Table 1.1), 200 μM of dNTPs (Invitrogen), and 1.2 pmol each of forward and reverse primers. The forward primer was modified at the 5' end by the addition of a fluorescent label (6-FAM, VIC, NED, or PET -Applied Biosystems). Labeled PCR products were

Table 1.1. Characteristics of microsatellite loci in *Corvus brachyrhynchos*

Locus	Repeat Motif	T_a (°C)	$MgCl_2$ (mM)	Allele Size Range (bp)	Primer Sequence (5'-3')	Primer Label	*n* (*n*)	N_A (N_A)	H_O	H_E	GenBank Accession No.
CoBr02	$(CA)_{17}$	58	1.5	140-167	F*: AGGGAAACGGCTGGAAAACTTG R: GCCCTCATCGATAGCGTACATATC	VIC	38 (172)	8 (9)	0.66	0.76	DQ528798
CoBr03	$(AC)_{21}$	53	1.5	148-166	F*:CAGATGTAAGACTTCTCAGCACCAG R: CCTGATTATTTTTAAGCCAACATTG	PET	36	6	0.67	0.78	DQ528794
CoBr06	$(CA)_{13}$	55	1.5	244-255	F*: CGGCAAACCCATGGAAATTAG R: GGGGGCAGATGGCATTATGT	PET	40 (163)	6 (7)	0.67	0.78	DQ528795
CoBr08	$(AC)_{14}$	50	1.5	208-220	F*: ATTGTCACCCATAACTTATAGACAG R: TATTCCAGCAGTAAATATTTAACCT	NED	40	5	0.58	0.71	DQ528796
CoBr09	$(TG)_{15}$	53	1.5	253-267	F*: AGGCCGATTTCTTGCTTTTC R: CAATGCCATCAGGACAAGTAAAC	NED	40 (164)	8 (9)	0.65	0.63	DQ528797
CoBr12	$(AC)_{20}$	59	1	86-114	F*: AGTCCCACTACCCTGCTCCATTTG R: AGGGGCTTGCTTGGGAGTTGA	PET	40 (59)	11 (14)	0.78	0.85	DQ528799
CoBr19	$(TTC)_{14}$	58	1.5	324-417	F*: CTATGGCCACCAAGGCAGAAATTC R: GTCTCAGCAGGGCACGGATGA	VIC	38 (172)	20 (23)	0.92	0.92	DQ528803
CoBr22	$(CTT)_{10}(CTCCTT)_5(CTT)_3$	54	1.5	118-159	F*: GTGAGTGAATTGACCAAAGAAGAT R: AGAGCATAGGAATGCAAAGATCT	FAM	38 (171)	12 (12)	0.82	0.84	DQ528802
CoBr24[+]	$(CTT)_6(TCTT)_{23}$	56	2	224-369	F*: GTAGGGGCAAAGGTGCTGAAA R: CCATGCATTGAGAAAACGAGAAA	FAM	40 (174)	29 (43)	0.70	0.95	DQ528800
CoBr25	$(AC)_{11}$	56	1.5	284-290	F*: TCCCAGAAGGCTCCAACATTATC R: CCAGGAGGCAAACAGCACTTAC	NED	38	4	0.53	0.64	DQ528793
CoBr36[+]	$(TCTT)_5(TTCT)_3(CTTT)_5$	53	2.5	197-286	F*: CTACTGCAGGAAATGCACCCATAC R: CCTTCAGTATATGCTCCCATAATG	FAM	39 (167)	24 (28)	0.74	0.94	DQ528801

* Indicates labeled primer. [+] Indicates loci with significant deviations from Hardy-Weinberg.

analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems), and allele sizes were estimated using a GeneScan™ 500 LIZ® size standard (Applied Biosystems) in the program GENEMAPPER™ version 3.7 (Applied Biosystems).

Results and Discussion

The 11 most variable loci were tested on an additional 25 unrelated birds to assess the variability at each locus and to determine the values of observed (H_O) and expected (H_E) heterozygosities. As part of a subsequent pilot study of genetic mating patterns, seven of these loci were used to genotype an additional sample of 126 related and unrelated individuals, and we discovered numerous additional alleles in this larger pool of individuals. The number of alleles per locus ranged from 4 to 43, with a mean of 13.8 (Table 1.1). Observed heterozygosities ranged from 0.25 to 0.92, and for most loci, the observed and expected heterozygosities were similar (Table 1.1). After Bonferroni correction for multiple comparisons, linkage disequilibrium was not detected between any pairs of loci (GENEPOP, Raymond and Rousset 2004). Null alleles were not detected at any of the loci using the program CERVUS 2.0 (Marshall et al. 1998). Due to a heterozygote deficiency, CoBr24 and CoBr36 deviated significantly from Hardy-Weinberg proportions. However, both CoBr24 and CoBr36 are the most variable loci and we suspect that the high allele: sample size ratios (e.g., 29 alleles in 40 individuals in CoBr24) are outside the parameter space for which this implementation of the test is appropriate. A BLAST search of these microsatellite flanking regions against the vertebrate sequences in GenBank identified no close matches, suggesting that none of the loci reported here are homologous to loci previously reported for other taxa. Genetic analyses using the 11 microsatellite loci described here will allow us to investigate the mating system of this socially-complex species, and to interpret their behavioral interactions in light of relatedness.

REFERENCES

Berg EC (2005) Parentage and reproductive success in the White-throated Magpie-jay, *Calocitta formosa*, a cooperative breeder with female helpers. Animal Behaviour, 70, 375-385.

Hoelzel AR (1992) Molecular Genetic Analysis of Populations: a Practical Approach. IRL Press at Oxford University Press, Oxford, NY.

Li Shou-H, Brown JL (2000) High frequency of extrapair fertilization in a plural breeding bird, the Mexican jay, revealed by DNA microsatellites. Animal Behavior, 60, 867-877.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology, 7, 639-655.

Quinn JS, Woolfenden GE, Fitzpatrick JW, White BN (1999) Multi-locus DNA fingerprinting supports genetic monogamy in Florida Scrub-jays. Behavioral Ecology and Sociobiology, 45, 1-10.

Raymond M, Rousset F (2004) GENEPOP (Version 3.4).http://wbiomed.curtin.edu.au/genepop/. Updated from Raymond, M. and Rousset, F. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 83, 248-249.

Stenzler LM, Fitzpatrick JW (2002) Isolation of microsatellite loci in the Florida Scrub-Jay *Aphelocoma coerulescens*. Molecular Ecology Notes, 2, 547-550.

Williams DA (2004) Female control of reproductive skew in cooperatively breeding brown jays (*Cyanocorax morio*). Behavioral Ecology and Sociobiology, 55, 370-380.

CHAPTER 2

REPRODUCTIVE PARTITIONING AND THE ASSUMPTIONS OF REPRODUCTIVE SKEW MODELS IN THE AMERICAN CROW*

---

* A. K Townsend, A. B. Clark, K. J. McGowan, and I. J. Lovette. 2009. Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crow. Animal Behaviour 77:503-512. Reprinted with permission from Elsevier .

Abstract

Understanding the benefits of cooperative breeding for group members of different social and demographic classes requires knowledge of their reproductive partitioning and genetic relatedness. From 2004-2007, we examined parentage as a function of relatedness and social interactions among members of 21 American crow (*Corvus brachyrhynchos)* family groups. Paired female breeders monopolized maternity of all offspring in their broods, whereas paired male breeders sired 82.7% of offspring, within-group auxiliary males sired 6.9% of offspring, and extragroup males sired 10.4% of offspring. Although adult females had fewer opportunities for direct reproduction as auxiliaries than males, they appeared to have earlier opportunities for independent breeding. These different opportunities for direct reproduction probably contributed to the male biased adult auxiliary sex ratio. Patterns of reproductive partitioning and conflict among males were most consistent with a synthetic reproductive skew model, in which auxiliaries struggled with breeders for a limited reproductive share, beyond which breeders could evict them. Counter to a frequent assumption of reproductive skew models, female breeders appeared to influence paternity, although their interests might have agreed with the interests of their paired males. Unusual among cooperative breeders, close inbreeding and incest occurred in this population. Incest avoidance between potential breeders did not significantly affect reproductive skew.

Introduction

Cooperative breeding in birds occurs when more than two individuals contribute to the care of young in a single brood. Although cooperative systems are highly variable (Brown 1987), cooperative groups in many species are characterized by a single breeding pair, assisted by (presumed) nonbreeding ‘auxiliaries,’ usually adult or

subadult offspring from previous broods. Potential benefits derived by auxiliaries (reviewed in Koenig & Dickinson 2004) include enhanced fitness of nondescendent kin (Hamilton 1964), prospecting for extragroup parentage (Young et al. 2007), and territory inheritance (Wiley & Rabenold 1984) or budding (Woolfenden & Fitzpatrick 1984). In some systems, molecular diagnoses of parentage have revealed that apparently nonbreeding auxiliaries also share in direct parentage (e.g. Rabenold et al. 1990). To understand the benefits of cooperative breeding for auxiliaries of different demographic classes, as well as their decisions to remain in their natal group or seek opportunities elsewhere, it is necessary to quantify reproductive opportunities within and outside of cooperative groups, as well as opportunities for independent breeding.

Reproductive skew theory provides a framework for predicting how parentage will be partitioned among same sex group members as a function of parameters such as relatedness, environmental constraints, group productivity and relative competitive ability of group members (reviewed in Clutton-Brock 1998; Johnstone 2000; Magrath & Heinsohn 2000; Reeve & Keller 2001; Magrath et al. 2004). In high skew groups, reproduction is concentrated in a subset of group members, whereas in low skew groups, reproduction is shared more equitably among group members. Different models of skew are built on different assumptions about interactions among group members (often referred to as dominants and subordinates), although the nature of these interactions is likely to vary among taxa. Models variously assume that (1) dominants have complete control over reproductive partitioning (e.g. Vehrencamp 1979; Vehrencamp 1983), (2) dominants have control only over group membership, whereas subordinates regulate their own reproductive share (Johnstone & Cant 1999), or (3) no individual has complete control over reproductive partitioning (Reeve et al. 1998). Also, most models assume that same sex group members decide their own reproductive partitioning (though see Cant & Reeve 2002), even though control by

opposite sex group members has been demonstrated in some cooperatively breeding birds (e.g. Williams 2004). Furthermore, current reproductive skew models assume an absence of incest avoidance, an assumption that might be violated in the nuclear family groups typical of many cooperatively breeding birds (Koenig & Haydock 2004). Incest avoidance could drive a pattern in which reproductive share decreases with relatedness of potential breeders, similar to the pattern predicted by concession models of reproductive skew (Emlen 1996; Magrath & Heinsohn 2000; Magrath et al. 2004). Numerous authors have called for tests of these assumptions as well as the predictions of reproductive skew models in the field as a useful approach to distinguishing among models (Clutton-Brock 1998; Johnstone 2000; Cant & Reeve 2002; Magrath et al. 2004).

The American crow (*Corvus brachyrhynchos*) is a broadly distributed North American corvid, with cooperatively breeding populations in which groups of up to 12 birds raise offspring (McGowan 2001; Verbeek & Caffrey 2002). These groups generally include a socially bonded pair of adults (henceforth 'breeders') and auxiliary birds that are usually offspring from previous broods (Caffrey 1992). Although this human commensal, readily observed species has been the subject of numerous behavioural studies (Kilham 1984; Chamberlain-Auger et al. 1990; Marzluff et al. 2001; Yorzinski et al. 2006), nothing has been reported about its genetic mating system, and relatedness among group members has never been quantified. The pair bonded breeders within cooperative crow groups appear socially monogamous (Verbeek & Caffrey 2002), but observations of extrapair copulation attempts suggest that crows might not be genetically monogamous (Kilham 1984; pers. obs.). Also, although long term monitoring in our population of banded birds suggests that most group members are first or second order kin, auxiliary individuals do sometimes immigrate into non-natal groups (Clark et al. 2006; pers. obs.).

Here, we described the genetic mating system of American crows in the context of their social and genetic group structure. Specifically, we (1) quantified behavioural differences between male breeders and auxiliaries, so that reproductive partitioning could be rigorously examined in terms of social role; and (2) described reproductive partitioning among birds within a family group as a function of their social role, age, sex and relatedness. In particular, we tested the hypothesis that reproductive skew differed when auxiliary birds were first order kin to the opposite sex breeder vs. when they were not. We predicted that skew would be greater when auxiliaries were first order kin to the breeder of the opposite sex because of incest avoidance. Because the concession reproductive skew model predicts a similar pattern without a role for incest avoidance, we then examined how the American crow system met the other assumptions of the basic reproductive skew models.

Methods

Study Area and Breeder Classification

From 2004-2007, we examined mating strategies and genetic group structure in a population of American crows in Ithaca, New York, which has been monitored continuously since 1989 (McGowan 1995, 2001; Clark et al. 2006). Auxiliaries in this population are both male and female, and individuals of both sexes help with antipredator vigilance, territory and nest defence (Serrell 2003; Wilson 2008), as well as provisioning the incubating females, nestlings and fledglings. The degree to which provisioning varies with auxiliary age, sex and relatedness to other group members is currently unknown. We collected behavioural and genetic information from 21 focal family groups occupying an area approximately 4 km^2. The study site included the Cornell University campus and adjacent natural areas, golf courses, shopping plazas and residential neighborhoods (described as ‘suburban’ in McGowan 2001). We

defined a crow ‘family group’ as a cohesive group of birds that maintained the same year-round all purpose territory among years, and that contained some of the same members from year to year. We monitored groups intensively from February-July (2-7 days per week) to document group membership, displacements, mate guarding, copulation, nest building, onset of incubation, hatching, provisioning and fledging. From August-January, we observed each group at least once per month to record membership and interactions among members.

We defined ‘auxiliaries’ as birds that shared the same territory with breeders throughout the breeding period; most auxiliaries helped provision offspring. We defined the ‘female breeder’ as the female that carried out almost all of the incubation and brooding (Kilham 1984; Chamberlain-Auger et al. 1990; Caffrey 2000). In our sample, classification of female breeders was unambiguous, because other females very rarely attempted to brood or incubate. We identified the ‘male breeder’ as the male that appeared dominant in interactions with all other group members (Kilham 1984) and that kept consistently closest to the female breeder from the start of the nest building period until the onset of incubation (Caffrey 1992) during our ad libitum observations (Martin & Bateson 1993).

The concept of extrapair paternity hinges on the presence of clear social pair bonds (defined in Westneat et al. 1990). Although male breeders appeared behaviourally distinct in our sample, and American crows generally have obvious social pair bonds (e.g. Kilham 1984; Caffrey 1992; Clark et al. 2006), we tested the repeatability of our social role assignments by determining whether or not the behaviours of male breeders were quantifiably distinct from the behaviours of male auxiliaries. In 2007, we conducted focal observations on 19 family groups during the nest building, egg laying and early incubation periods (ending observations by the second day of incubation). Using the ‘breeder’ and ‘auxiliary’ classifications that we

had generated from our ad libitum observations, we compared the level of pair behaviours between female breeders and male breeders vs. between female breeders and male auxiliaries. During 1-4 focal observations per family group (25-150 minutes per focal observation, depending on how long a family group could be followed on a given day), we recorded all displacements (i.e. one individual supplanting another), allopreens and copulation attempts. At 10 minute intervals within these focal observations, we estimated the distance of each bird in the group from the female breeder. When a bird flew out of sight, we conservatively estimated its distance from the female breeder as the furthest distance we could see in that habitat. In some intervals we were uncertain of the location of some adult males relative to the female breeder; we did not include these intervals in our analyses.

Genetic Sampling and Analyses

On days 24-30 after hatching, we climbed to each nest to mark nestlings with unique combinations of metal bands, colour bands and patagial tags. We collected blood (~150 ul) from the brachial vein of live nestlings, and collected tissue samples from dead nestlings in and under these nests. By the 2007 field season, 98 of 125 adult birds (78.4%) in our 21 focal groups were banded or identifiable by unique scars or other conspicuous deformities. During most breeding attempts, there was not more than one unbanded or unscarred individual in each group (mean number of unmarked birds per breeding attempt + SE = 1.19 + 0.09; range 0-2). We extracted DNA from blood or feathers from 124 of the 125 marked and unmarked adult birds in these groups. Many had blood samples drawn when they were banded as nestlings. From the remaining adults, we collected passively moulted feathers while they were provisioning nestlings or fledglings on their territories (June-August). Unmarked birds present in multiple years were regenotyped using new feathers collected each year to reconfirm their

identity. One auxiliary, which was present in a single year, disappeared before it could be sampled. Yearlings that had not been banded as nestlings could be distinguished from adults (defined here to include birds two years and older) by plumage: yearling crows have browner feathers and more pointed rectrices than adult birds until their definitive prebasic moult at 15 months (Emlen 1936).

DNAs were extracted from blood samples using Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY, U.S.A.) and from feather tips using DNeasy tissue kits (Qiagen, Valencia, CA, U.S.A.) following the manufacturers' protocols. We sexed all individuals at diagnostic sex linked alleles, using the 2550/2718 primer set (Fridolfsson & Ellegren 1999). We genotyped offspring and family members at 12 polymorphic microsatellite loci, selected from a panel that we previously developed for American crows (Schoenle et al. 2007) and from another panel isolated from the Mariana Crow (*Corvus kubaryi*; Tarr & Fleischer 1998). The forward primer of each pair was labeled using the fluorescent dyes 6-FAM, NED, PET, or VIC (Applied Biosystems, Foster City, CA, U.S.A.), and polymerase chain reaction (PCR) was carried out with optimized conditions and reagents (Table 2.1). Genotyping was performed on a 3100 Genetic Analyzer (Applied Biosystems). All alleles were scored automatically and confirmed visually using GENEMAPPER^{TM} version 3.7 software (Applied Biosystems). To validate the reliability of moulted feathers as a DNA source, we compared genotypes of 30 colour marked individuals from which we had both feathers and blood samples. In all comparisons, genotypes derived from these two DNA sources were identical.

We used the maximum likelihood approach used for parentage analyses in the program CERVUS 3.0 (Kalinowski et al. 2007). We specified a potential typing error of 1%, and specified the proportion of sampled candidate parents at 90% to account for unsampled adults in areas adjacent to our focal territories. We specified relatedness

among 5% of candidate parents at 0.5 to account for kinship of potential breeders within family groups. Two loci (CoBr24 and CoBr03) had high frequencies of inferred null alleles and were not included in the final analyses. The ten remaining loci provided a powerful marker set for parentage discrimination, with a mean allele frequency of 13.7 alleles/ locus, combined exclusion probabilities of 0.99915 when neither parent was known, and 0.99998 when one parent was known. Allele frequencies at these ten loci did not deviate significantly from Hardy-Weinburg expectations.

Shared maternity might occur (albeit rarely) in this American crow population, as suggested by a 2001 observation of an exceptionally large clutch incubated by multiple females (K. J. McGowan, unpublished data). Although no clutches were incubated by multiple females in our sample, we tested the assumption that female breeders were the mothers of all nestlings in their nests before assessing paternity. We first used CERVUS to identify mothers (with no 'known parent' specified), including all sampled females (yearlings and adults) present in each year as potential mothers. For 192 of 202 offspring, the female breeders of their respective broods were scored as the most likely candidate parents at or above the 95% confidence level. In the remaining ten cases, older siblings of these offspring were selected as the most likely candidate mother, with the female breeders selected as the second most likely candidate mother. This result was not unexpected, because this comparison does not incorporate information on the mate's genotype and therefore has weak power to exclude closely related individuals. In all such cases, however, there were no mismatches between either the sibling-offspring or breeder-offspring dyads. In eight of these ten cases, the selected sister-auxiliary was a yearling and therefore likely to be sexually immature (Black 1941), implicating the breeder as the actual mother. To examine further whether the female breeder or the sister-auxiliary was the most likely

candidate, we used a protocol newly implemented in CERVUS 3.0 to run a 'parental pair' analysis (sexes known) for these ten offspring, now including all sampled males and females (both yearlings and adults) present in each year as candidate parents. A parental pair analysis seeks the most likely parental pair when neither parent is known. In all ten cases, the expected male and female breeders were selected as the most likely candidate parents; the auxiliary females selected in the simple dyad analysis were not selected. Considered together, these analyses strongly support the assumption that female breeders were the mothers of all nestlings in their nests. When there were allelic mismatches between female breeders and their putative offspring, we regenotyped both members of the dyad to check for typing error. In our final sample, there were only two allele mismatches in all mother-offspring comparisons (out of N = 202 dyads and N = 4040 pairwise comparisons of alleles), which might be attributable to mutation (Slate et al. 2000).

We then examined paternity, specifying female breeders as 'known parents,' and including all sampled adult males present in a given year as potential fathers. Confidence levels of 80% or greater might be sufficient to identify true genetic parents when combined with behavioural data (Slate et al. 2000). We therefore accepted males suggested by CERVUS 3.0 as true sires when (1) they were selected as the most likely candidate at the 95% confidence level or above (N = 117); (2) they were selected at the 80% confidence level and had no allelic pair mismatches (N = 62); or (3) the male breeder was selected at the 80% confidence level, with a single allelic pair mismatch (N = 7). When the male breeder was not the suggested sire and the confidence level for the suggested candidate fell below 95%, we denoted those offspring as having extrapair sires of unknown identity (N = 17). None of the proposed candidates in these latter 17 cases were auxiliary males within the family group of the respective offspring, and in each case, all adult males in the group had been sampled; the

extrapair sires were therefore further described as ‘extragroup.’ In four cases, candidate fathers selected at the 95% confidence level were sons of the male and female breeder (i.e. mother-son incestuous matings). In each case, there were no pair or trio mismatches between the incestuous candidate sire and putative offspring, whereas the male breeder shared 2-3 mismatches with that offspring.

We used our microsatellite genotypes to generate pairwise genetic relatedness coefficients between all pairs of family members using the program RELATEDNESS v.5.0.8 (Queller & Goodnight 1989). Negative coefficients suggest that two individuals are less related than expected by chance if the two genotypes were randomly selected, whereas positive coefficients suggest that the individuals are related (e.g. mean coefficients of 0.5, 0.25 and 0.125 are expected between first, second and third order kin, respectively). In accordance with these expectations, preliminary results showed a mean + SE coefficient of relatedness between female breeders and their putative offspring of 0.52 + 0.009 (N = 156).

Statistical Analyses

Statistical analyses were conducted in JMP version 7.0, using nonparametric tests when variables deviated from normal distributions. To compare the level of mate guarding between males that we had qualitatively classified as ‘breeders’ or ‘auxiliaries,’ we compared the mean distance of the male breeder vs. the mean distance of the closest adult male auxiliary from the female breeder in a given family group (Wilcoxon signed-ranks test, with pairing done by family group), averaged over all 10 minute intervals of our focal observations. In certain analyses of group structure (specified in the Results), we tested information from only a single, arbitrarily selected year (2007) from each family group to avoid analysing the same individuals repeatedly. All statistical tests were two-tailed.

To estimate reproductive skew, we calculated the binomial skew index (B) in Skew Calculator 2003 v 1.2.1. (Nonacs 2000, 2003), which corrects observed with expected variance in reproductive success of each group member, with the null expectation that each member has an equal probability of reproduction. Positive B values indicate that observed skew is greater than expected by random chance, whereas zero or negative values indicate that parentage is randomly or more equally shared than expected by chance, respectively, from a simulated random distribution. Only adult birds were included as potential breeders. Offspring sired by extragroup males were not included in the analysis, as skew indices apply only to reproductive partitioning within groups.

Ethical Note

All capture, handling, marking, observation and blood sampling of American crows was carried out under permits from the U.S. Geological Survey Bird Banding Lab (#22263) and New York State (#33), and under protocols approved by the Binghamton University (# 537-03 and 607-07) and Cornell University (# 1988-0210) Institutional Animal Care and Use Committees.

Results

Classification of Individuals by Dominance and Mating Behaviour

To test whether our 2004-2007 qualitative classifications of male social role based on ad libitum behavioural observations were supported by quantitative focal observations of pair behaviours, we observed 19 family groups for 80.9 hours between 17 March and 7 April 2007 (mean + SE = 3.32 + 0.24 focal observation periods per group, N = 63 focal observations). There was more than one adult male in 13 of these 19 family

groups. In comparisons of within-group male breeders to male auxiliaries, we limit our sample to these 13 groups.

Males that had been previously classified as breeders were behaviourally distinct from auxiliary males. In the 13 groups with more than one adult male, male breeders were likely to be closer to the female breeders than adult male auxiliaries (Wilcoxon signed-ranks test: $T = 45.5$, $N = 13$, $P = 0.0001$): they were the closest adult male to the female breeders in 299 of 359 (83.3%) intervals, whereas adult male auxiliaries were closer in only 20 of 359 (5.5%) intervals; breeders and adult male auxiliaries were equidistant from the female breeder in 40 of 359 (11.1%) intervals. Seven of the 19 male breeders were allopreened by female breeders, whereas adult male auxiliaries were not observed being allopreened. Male breeders displaced other birds a mean + SE of 0.83 + 0.22 times per hour and were not displaced by other birds of any sex or age.

From 2004-2007, we observed six copulations between male and female breeders from five different family groups. In each case, the male breeder approached the female from the front and she performed precopulatory displays (described in Kilham 1984; Verbeek & Caffrey 2002), lowering herself to the ground and quivering her wings. Females appeared passive and receptive during these copulations. During one within pair copulation, the female uttered loud, monotonous calls, but in all other cases, the females were silent. These within-pair copulations were observed approximately seven days prior to the onset of incubation until the first day of incubation. We observed seven extrapair copulation attempts by within-group auxiliary males from five family groups; six attempted copulations by extragroup males in four family groups; and one additional copulation attempt by an unidentified extrapair male. All 14 attempted extrapair copulations were interrupted by the male breeder. Females did not perform precopulatory displays prior to these copulation

attempts and appeared to actively resist them, flapping their wings throughout and vocalizing loudly. Extrapair males were observed to (1) approach a female breeder from behind and attempt to mount her while she foraged (N = 1); (2) drop on her while she was collecting nest material (N = 2); (3) chase her below her nest tree and pin her to a branch (N = 1); and (4) land on her while she was incubating (N = 10). In four of these ten extrapair copulation attempts with incubating females, multiple (4-6) birds landed on the incubating female simultaneously. Extrapair copulation attempts were observed approximately 12 days prior to the onset of incubation until the fourth day of incubation. Three within-group auxiliary males that were observed in these apparently unsuccessful copulation attempts did ultimately attain paternity in that brood with their respective female breeders (Table 2.1).

Table 2.1. Optimized conditions for microsatellite loci

Locus	Annealing temp (^{o}C)	$MgCl_2$ concentration (mM)	# Alleles	Expected heterozygosity (%)
CoBr22	57	3.75	15	0.88
CoBr02	57	3.75	9	0.76
CoBr19	57	3.75	24	0.92
CoBr36	57	3.75	36	0.93
CoBr12	57	3.75	13	0.82
CoBr06	55	1.5	6	0.71
CoBr25	55	1.5	4	0.57
CoBr08	55	1.5	5	0.59
CoBr24*	57	3.75	48	0.95
CoBr03*	55	1.5	7	0.72
Ck1B6G	55	1.5	11	0.86
Ck5A5F	55	1.5	14	0.87

*Loci not used in final analyses because of high inferred null allele frequency.

Relatedness and Group Structure

The mean + SE size of the focal groups at the beginning of the breeding period was 4 + 0.22 adults, $N = 60$ breeding attempts (range = 2-9 birds; Fig. 2.1; note that these summary statistics include multiple years of data from most groups). This distribution of group sizes was very similar to the distribution described in this population from 1989-1999 (McGowan 2001). There were 48 unique male and 26 unique female auxiliaries in these groups from 2004-2007. Considering only a single year (2007) from each family group to avoid analyzing the same individuals over time, there was no difference in the number of yearling male and female auxiliaries (Wilcoxon signed-ranks test: $T = -4.0$, $N = 21$, $P_{\text{two tailed}} = 0.78$; mean + SE = 0.76 + 0.22 males and 0.71 + 0.18 females per family group); there were, however, significantly more adult male than female auxiliaries (Wilcoxon signed-ranks test: $T = -37.0$, $N = 21$, $P_{\text{two tailed}} = 0.017$; mean + SE = 1.19 + 0.25 males and 0.43 + 0.15 females per family group; Fig. 2.2). Overall, male auxiliaries were significantly older than female auxiliaries (Mann-Whitney U test: $U = -2.24$, $N_1 = 22$, $N_2 = 39$, $P = 0.025$; mean + SE = 2.28 + 0.25 years for males and 1.45 + 0.13 years for females). This latter result is congruent with a previous report showing that males are older than females in the same social class (Clark et al. 2006). From 2004-2007, five female breeders and only one male breeder died or disappeared from the 21 focal groups.

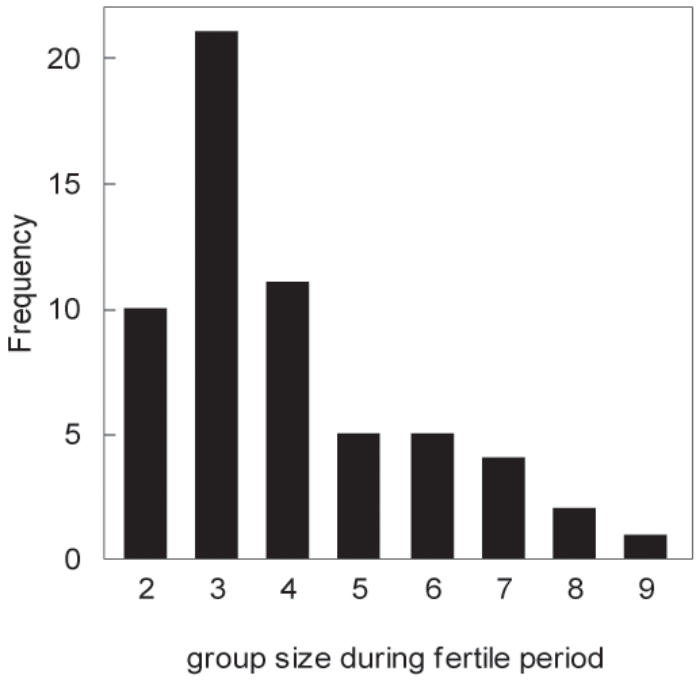

Figure 2.1. Frequency distribution of group sizes at the beginning of each breeding attempt (N = 60 breeding attempts).

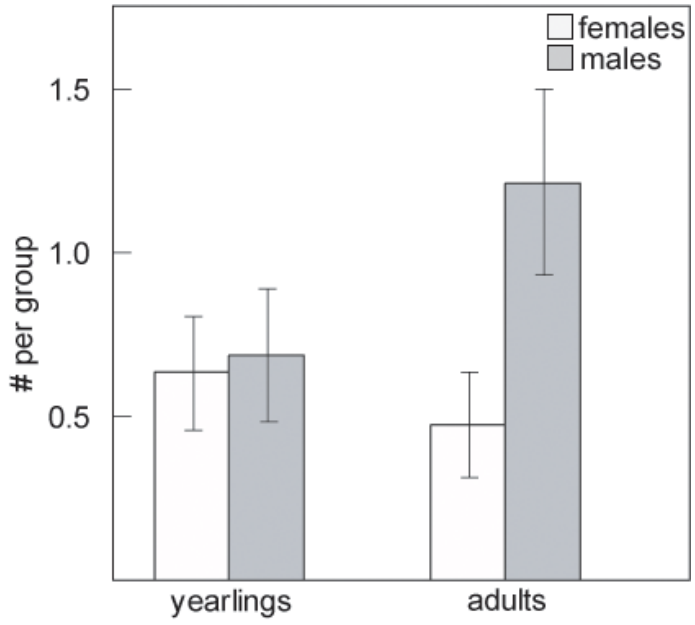

Figure 2.2. Mean number of auxiliaries per group + SE by age class and sex. Only one year of data (2007) is shown to avoid analysing birds repeatedly across years.

We genotyped 24 breeding pairs from our 21 family groups, because we collected DNA from three of the pairs that changed over the course of this study. The mean relatedness coefficient between male breeders and their paired female breeders was close to zero (mean + SE = 0.03 + 0.04, N = 24 dyads, range -0.31-0.38), although six of these 24 dyads (25.0%) appeared to be related at the level of second or third order kin (i.e. with coefficients of relatedness > 0.125; Fig. 2.3). Dyads of female breeders and adult male auxiliaries within a family group shared a lower mean coefficient of relatedness than dyads of male breeders and adult male auxiliaries in that group (Paired t test: t_{16} = -2.75, P = 0.014). Sixteen adult male auxiliaries unrelated to the female breeder vs. three adult male auxiliaries unrelated to the male breeder were distributed among 11 and 2 different family groups, respectively. Adult male auxiliaries unrelated to the breeders occurred for three reasons, which were not mutually exclusive: (1) the disappearance and replacement of female breeders in three groups, (2) the presence of nondescendent kin of the male breeder in seven groups, and (3) the immigration of three males, unrelated to either breeder, into two groups.

Genetic Parentage and Reproductive Skew

We genotyped 202 offspring from 60 broods (mean + SE = 3.36 + 0.19 offspring per brood, range = 1-6 offspring), belonging to 21 family groups (2.86 + 0.19 broods per family group, range = 1-4 broods). Of these offspring, 35 (17.3%) were sired by males other than the social breeder. Extrapair offspring were distributed in 17 (28.81%) different broods, among 13 of the 21 family groups (61.9%).

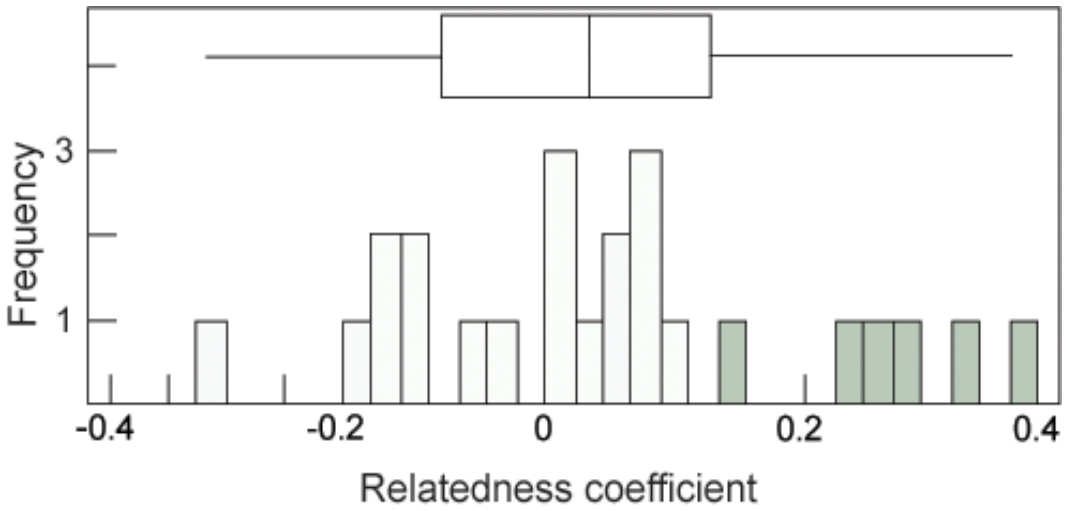

Figure 2.3. Frequency distribution of pairwise related coefficients between male and female breeders. Gray bars indicate relatedness coefficients at or above the level predicted for third-order kin (r > 0.125). The median coefficient of relatedness, shown by the box-and-whiskers plot, is 0.015.

Extragroup males sired 21 offspring (10.4%), distributed in 13 broods among 11 family groups. Five of these 21 offspring were sired by male breeders from adjacent territories (N = 3 adjacent male breeders), whereas 16 were sired by extragroup males of unknown identity. Auxiliaries in this sample did not obtain parentage outside of their family group. Within-group auxiliary males sired 14 offspring (6.9%), distributed in seven broods among six family groups. Auxiliary sons incestuously sired four offspring (2.0%), stepsons sired six offspring (3.0%), and nondescendent kin of the male breeders sired four (2.0%) of the 202 offspring (Table 2.2). All auxiliaries that gained paternity were related to the male breeder; the three unrelated auxiliary males did not gain paternity. Yearling birds did not achieve parentage, supporting our behavioural observations that suggest they are sexually immature until they are two years old.

Table 2.2 . Pairwise relatedness coefficients and apparent relationships between the six within-group subordinate sires and their male breeders and females

Extrapair sire #	Relatedness between extrapair sire and female breeder	Relatedness between extrapair sire and male breeder	Extrapair sire's apparent relationships
1	-0.0656	0.2574	half-brother of male breeder; rose in rank during breeding season*
2	0.1557	0.4908	son of male breeder; fertilized stepmother*
3	0.0471	0.4698	son of male breeder; fertilized stepmother*
4	-0.1973	0.4375	brother of male breeder
5	0.5811	0.6326	son of male and female breeder; fertilized mother
6	0.5918	0.5814	son of male and female breeder; fertilized mother

*Resisted copulation attempts observed between extrapair sire and female breeder; see details in text.

The mean value for the binomial skew index B + SE was 0.18 + 0.06 (N = 10 groups, range = -0.02-0.58) among males that were not first order kin of the female breeder and 0.21 + 0.06 (N = 9 groups; range = 0.03-0.53) when auxiliary males were first order kin of the female breeder. Reproduction was significantly skewed among males of both relatedness classes ($p<0.001$). Skew was not different between male breeders and auxiliary males that were related and unrelated to the female breeder (t test: $t_{17} = 0.46$, $P = 0.65$).

Discussion

American crow groups in this population were characterized by paired male and female breeders that were behaviourally distinct from auxiliary birds. During the nest building and early incubation period, male breeders were usually the closest adult male to the female, displaced other birds (particularly adult male auxiliaries) but were not themselves displaced, and disrupted copulation attempts by other males but themselves copulated undisturbed. Reproduction was skewed towards the paired breeders in both sexes. Female breeders monopolized all maternity within their broods, whereas male breeders sired 82.7% of the offspring in their broods, within-group auxiliary males sired 6.9% of offspring and extragroup males sired 10.4% of offspring. Focal auxiliaries did not sire offspring in neighboring broods, suggesting that prospecting for extragroup parentage (Young et al. 2007) was not important for any class of auxiliary. It is possible, however, that offspring sired by unknown extragroup males were sired by adult male auxiliaries outside of the focal family groups. Almost all auxiliaries were relatives of the male breeders, either as offspring from previous broods or nondescendent kin (e.g. nephews, brothers). Only three of 48 auxiliary males were unrelated to male breeders, and they did not contribute to direct reproduction. Contrary to our prediction, reproduction was not significantly more skewed towards male breeders when the auxiliaries were first order kin of the female breeders.

A substantial proportion (25.0%) of paired birds shared coefficients of relatedness greater than the level of third order kin, and a small proportion of offspring (2%) were sired incestuously through fertilizations of mothers by their adult sons. Proximately, inbreeding in this population might have been promoted by delayed dispersal and short natal dispersal distances of many individuals of both sexes (mean dispersal distance + SE for females = 5.2 + 1.81 km, range =0.04-59.6, N = 33; for

males = 0.85 + 0.29 km, range =0-7.8, *N* = 27; K. J. McGowan, unpublished data). Although true mean natal dispersal distance was likely underestimated (birds that stayed close to home were more detectable), it was clear that many individuals, both male and female, did not disperse far from their natal territory to breed.

The ubiquity of female choice of their extrapair partners in birds has been questioned (Arnqvist & Kirkpatrick 2005; Eliassen & Kokko 2008), particularly as direct observations of extrapair copulations are rare (Westneat & Stewart 2003). Our data suggest that female American crows might not have complete control over their extrapair reproductive partners. The six within-pair copulations that we observed were all solicited by female breeders, whereas the fourteen extrapair copulation attempts that we observed (both by auxiliary and extragroup males) appeared to be resisted by female breeders; these observations were congruent with copulatory behaviour described in a Florida population of American crows (Kilham 1984), as well as in the congeneric Northwestern crow (*Corvus caurinus;* Verbeek & Butler 1999). Several (but not all) of the extrapair males that we observed attempting resisted copulations gained paternity with these female breeders. Whether or not forced copulations can lead to fertilizations in birds that lack an intromittant organ is controversial (Gowaty & Buschhaus 1998; Westneat & Stewart 2003), and we do not know if these males successfully gained paternity during a resisted copulation attempt, or if females solicited copulations from them (Double & Cockburn 2000) or submitted to these copulations to reduce the costs of harassment (Arnqvist & Kirkpatrick 2005; Eliassen & Kokko 2008) at other times. Even when females do appear to resist copulations, they might be selecting males that can overcome their resistance (Kokko et al. 2003), or attempting to increase mate guarding by their consort males while encouraging copulation attempts by other males (Westneat & Stewart 2003; Pradhan et al. 2006).

In support of the idea of female control over extrapair fertilizations, the five extragroup offspring with identified sires were all acquired by neighboring breeders (not auxiliaries). As an argument against the idea of complete female control, however, four offspring were sired by adult auxiliary sons of the female breeders; such incestuous fertilizations are unlikely to be in the interest of female breeders (Emlen 1996).

Group Composition and Opportunities for Direct Reproduction

The sex ratio of adult auxiliaries was male biased and male auxiliaries were older, on average, than female auxiliaries. The observed adult auxiliary sex ratio and age structure might have been influenced by gender differences in opportunities for direct reproduction within and outside natal groups. Adult male auxiliaries (even those related to female breeders) did occasionally sire offspring in their group as early as their second year, whereas female auxiliaries did not contribute at any age. Females in this population, however, bred independently as early as their second year (possibly facilitated by the relatively high rate of death and disappearance of female breeders in this population; McGowan 2001; this paper), whereas in the 19 years that this population has been monitored, marked males have not bred independently until at least their third year (K. J. McGowan, unpublished data). More opportunities for early independent breeding, combined with fewer opportunities for direct reproduction within their natal groups, could thus create incentives for adult auxiliary females to leave their natal group earlier than auxiliary males.

The auxiliary sex ratio in cooperatively breeding birds is typically biased towards males (Williams & Rabenold 2005). In our population, the adult auxiliary sex ratio was male biased, whereas the sex ratio of yearlings was unbiased. Our behavioural and parentage data suggested that yearlings are sexually immature, which

might be characteristic of the genus (reviewed in Caffrey 1992). The absence of variation in reproductive opportunities between the sexes probably contributed to our unbiased yearling sex ratio. However, the sex ratio of yearling auxiliaries in a California population of American crows (*Corvus brachyrhynchos*) was female biased (N = 29 yearlings), even though these yearlings also appeared sexually immature (Caffrey 1992). The factors driving the unusual female biased sex ratio in this population were unclear (Caffrey 1992), although it is apparent that opportunities for immediate, direct reproduction cannot explain all variation in auxiliary sex ratio biases in the American crow.

Predictions and Assumptions of Reproductive Skew Models

The two basic categories of skew models (reviewed in Magrath et al. 2004) are sometimes referred to as 'transactional' (including 'concession' and 'restraint' models) and 'tug-of-war' models. In concession models, a dominant allows a subordinate the minimum share of reproduction that will compensate it for remaining in the group (Vehrencamp 1979, 1983). In restraint models, a subordinate limits its own share of reproduction to a level above which it will be evicted by the dominant (Johnstone & Cant 1999). In contrast, in tug-of-war models, reproductive share is based on the relative competitive ability of dominants and subordinates, without regard for group stability (Reeve et al. 1998). Concession models predict that reproductive skew will increase with relatedness, restraint models predict that reproductive skew will decrease with relatedness, whereas tug-of-war models variously predict that skew will increase with (Cant 1998), decrease with, or else be unaffected by relatedness (Reeve et al. 1998), depending on the how dominance is described (Beekman et al. 2003) or reproductive share gained (reviewed in Johnstone 2000). Within this simplified framework, the pattern of reproductive partitioning and

male relatedness that we observed in American crows was most consistent with restraint and certain tug-of-war models: only auxiliaries related to the male breeder shared in reproduction. We can gain further insight about the factors that might (and might not) influence reproductive partitioning in male American crows by considering behavioural information on the nature of their interactions in light of the assumptions of the different reproductive skew models.

Assumption 1: Control over reproductive partitioning and group membership. Reproductive skew models vary in their assumptions about who controls same sex reproductive partitioning and group membership. Concession models assume that a dominant individual has perfect control over reproductive partitioning, and that subordinate individuals leave the group voluntarily if they have larger fitness payoffs elsewhere (Vehrencamp 1979, 1983), whereas restraint models assume that subordinates have control over their reproductive share, but can be forcibly ejected or excluded by the dominant to prevent or limit their reproductive share. In contrast, tug-of-war models assume that neither subordinates nor dominants have complete control over reproductive partitioning, and make no explicit assumptions about control over group membership (Reeve et al. 1998).

Our observations suggest that American crow male breeders did not have complete control over reproductive partitioning, but had the ability to forcibly evict auxiliaries. Incomplete control over the fertilizations of their females was suggested by the occurrence of extragroup paternity, which is unlikely to have any benefit for male breeders. Incomplete control by either the breeders or auxiliary males over reproductive shares was further suggested by the observed auxiliary copulation attempts, all of which were interrupted by male breeders, suggesting that there was continual conflict over reproductive share. Some, but not all, of these auxiliaries

successfully secured a reproductive share. Control by male breeders over group membership, however, was suggested both by the low incidence of auxiliaries unrelated to male breeders and by an observation of a three year old male in the process of emigrating into a non-natal group. He first spent several days attempting to join one non-natal group, where he was repeatedly attacked by the male breeder. He was then accepted into another non-natal group without apparent conflict. This evidence for incomplete control over reproductive partitioning, combined with breeder control over group membership, was most consistent with the assumptions of synthetic models of tug-of-war and restraint (but not concession), in which male auxiliaries struggled with breeders over a reproductive share in a 'window of selfishness' (sensu Reeve 2000) above which the subordinate could be evicted (Johnstone 2000; Reeve 2000; Magrath et al. 2004).

Most models of reproductive skew, including the synthetic models that might best predict reproductive partitioning among American crow males, assume that decisions about reproductive partitioning are limited to same sex group members. In some systems, however, decisions about reproductive partitioning among males are made by females, which appeared to be the case in another cooperative corvid, the brown jay (*Cyanocorax morio*; Williams 2004). This intersexual control over reproductive skew can confound interpretations of classical skew models (Magrath & Heinsohn 2000; Magrath et al. 2004). Our observations suggested that female American crows might have had some influence over reproductive partitioning among males, but that female breeders reinforced the reproductive share of their pair males: females appeared to resist fertilizations outside of their pair bond. When the interests of female and male breeders are in agreement, the predictions of classical skew models are unchanged (Cant & Reeve 2002).

Assumption 2: No incest avoidance. Current models of reproductive skew do not account for potential incest avoidance among group members, which might have a major effect on reproductive partitioning in nuclear families (Emlen 1996; Magrath & Heinsohn 2000). If matings between relatives are avoided (a pattern that appears frequently, but not universally true in cooperatively breeding birds; Koenig & Haydock 2004), a pattern of reproductive partitioning will emerge in nuclear families in which reproductive share is lower for related birds, consistent with concession models of skew (Magrath & Heinsohn 2000). When there is strong evidence for incest avoidance in a given system (e.g. Koenig et al. 1998), one might reasonably exclude incestuous pairs from the set of potential breeders (Magrath et al. 2004). The assumption of incest avoidance is not appropriate to all systems, however, as demonstrated in this American crow population. These crows did not completely avoid incest and inbreeding, and reproduction was not significantly more skewed towards male breeders when auxiliaries were first order kin of the female breeders. This latter result might have been influenced by the small sample size of groups, as well as the high degree of overall skew. None the less, we can conclude that, in our sample of American crows, incest avoidance did not create a pattern in which skew increased with relatedness. The occurrence of incest in cooperatively breeding birds, however, appears to vary even among the cooperative corvids. Although incest was detected in relatively small samples of American crows (this study) and brown jays (Williams & Rabenold 2005), incest appears absent in the carrion crow (*Corvus corone corone*; Baglione et al. 2002) and extremely rare in the Florida scrub-jay (*Aphelocoma coerulescens*; Quinn et al. 1999). The importance of incest avoidance in driving patterns of reproductive partitioning must therefore be assessed separately in each system.

Comparison with Other Cooperative Corvids

Out of the 26 known cooperative jays and crows (Ligon & Burt 2004), the six species with described genetic mating systems exhibit wide variation in both social mating systems and patterns of reproductive partitioning (Table 2.3). For example, the proportion of mixed paternity broods in two cooperative *Aphelocoma* jays ranges from among the highest reported in birds (63% of broods in the plural breeding Mexican Jay, *Aphelocoma ultramarina*; Li & Brown 2000) to the lowest (0% of broods in the monogamous Florida scrub-jay; Quinn et al. 1999). Likewise, mixed maternity within single broods occurs with apparent regularity in the white-throated magpie-jay (*Calocitta formosa;* Berg 2005), but rarely (Quinn et al. 1999; Baglione et al. 2002) or inconsistently (Lawton & Lawton 1985; Williams 2004) in the other corvids. Even when the proportion of polygamous broods appeared superficially similar across taxa, as exhibited by congeneric American crows and carrion crows (Baglione et al. 2002), the identity of the auxiliary sires and their interactions with the male breeders appeared to differ substantially. In the American crow, four of the six reproducing auxiliaries bred on their natal territories, whereas in the carrion crow, shared reproduction appeared to be limited to non-natal immigrants. Aggressive interactions among potential male breeders were obvious in American crows, but not in carrion crows (Baglione et al. 2002).

Table 2.3. Described variation in social and genetic mating systems among cooperative corvids

Species	Social mating system	% Polyandrous broods	Mixed maternity	Source
Florida scrub-jay *Aphelocoma coerulescens*	Monogamous	0%	Rare	Quinn et al. 1999
carrion crow *Corvus corone corone*	Monogamous or polyandrous	26%	Rare	Baglione et al. 2002; pers. comm. V. Baglione[1]
American crow *Corvus brachyrhynchos*	Monogamous	28.8%	No	This study
brown jay *Cyanocorax morio*	Plural breeding; joint nesting; polygynandrous	31-43%	Yes[1]; but see Williams 2004	Lawton & Lawton 1985[1]; Williams 2004; Williams & Rabenold 2005[2]
white-throated magpie-jay *Calocitta formosa*	Monogamous[1]	33.3%-61.5%	Yes	Langen 1996; Berg 2005; pers. comm. J. Ellis[1]
Mexican Jay *Aphelocoma ultramarina*	Plural breeding; monogamous	63.0%	No	Li & Brown 2000

Describing a single pattern of reproductive partitioning for an entire species can be misleading, because patterns of reproductive partitioning vary even within populations over time. Evidence of joint nesting in a population of brown jays (Lawton & Lawton 1985), for example, was absent in the same population in subsequent studies (Williams 2004). Such variation in patterns of reproductive partitioning might be partly driven by environmental conditions. For example, reproductive skew in a population of cooperatively breeding white-winged choughs (*Corcorax melanorhamphos*) decreased after a drought disrupted previously stable

nuclear family groups (Heinsohn et al. 2000). American crows have also experienced a recent change in environmental conditions in the form of West Nile virus, which elevated breeder mortality in 2002-2003 in the Ithaca population (Clark et al. 2006). Although we have not yet examined patterns of reproductive skew before and after the epidemic, it might have led to an increase in population level reproductive skew, as predicted by the restraint model (Johnstone & Cant 1999), if it lowered ecological constraints by creating more opportunities for independent breeding.

Recent emphasis has been placed on synthesizing reproductive skew models into a universal model applicable to many group-living species (e.g. Reeve & Shen 2006; Buston et al. 2007). A single model predicting reproductive partitioning among corvids would need to accommodate variation in important processes contributing to these patterns, such as incest avoidance and intersexual control. A thorough comparison of the factors influencing reproductive skew across more corvid populations and species, with careful attention to both the assumptions and predictions of the different skew models, might illuminate the most important and general processes driving patterns of reproductive skew among them. Such comparative approaches have been taken for the social insects (Reeve & Keller 2001) and primates (Kutsukake & Nunn 2006). The application of this approach to taxa as disparate as corvids, primates and insects might provide insights to the factors of the most universal importance in predicting patterns of reproductive skew.

In conclusion, our observations of reproductive partitioning in the American crow are most consistent with a synthetic skew model (Johnstone 2000) of tug-of-war and restraint (but not concession), in which male auxiliaries struggle with breeders over a reproductive share in a ‘window of selfishness’ (Reeve 2000), beyond which they will be evicted. Counter to a frequent assumption of reproductive skew models, females did appear to influence paternity, although they might have reinforced the

interests of their mates (Cant & Reeve 2002). Inbreeding and incest did occur in this population, and incest avoidance between potential breeders did not significantly affect skew in our sample. It would be useful, however, to create and test predictions of a model that incorporates potential costs of inbreeding on optimal skew (e.g. in terms of lower group productivity; Johnstone 2000; Magrath et al. 2004) in a larger sample. Also, a complete test of reproductive skew in American crows should ultimately consider many additional factors, such as ecological constraints on independent breeding, relative competitive ability of group members (Beekman et al. 2003), the degree to which one or more auxiliaries influence reproductive productivity (Johnstone et al. 1999) and relatedness asymmetry among potential breeders (Reeve & Keller 1996).

REFERENCES

Arnqvist, G. & Kirkpatrick, M. 2005. The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. *American Naturalist*, **165**, S26-S37.

Baglione, V., Marcos, J. M., Canestrari, D. & Ekman, J. 2002. Direct fitness benefits of group living in a complex cooperative society of carrion crows (*Corvus corone corone*). *Animal Behaviour*, **64**, 887-893.

Beekman, M., Komdeur, J. & Ratnieks, F. L. W. 2003. Reproductive conflicts in social animals: who has power? *Trends in Ecology & Evolution*, **18**, 277-282

Berg, E. 2005. Parentage and reproductive success in the white-throated magpie-jay, *Calocitta formosa*, a cooperative breeder with female helpers. *Animal Behaviour*, **70**, 375-385.

Black, C. 1941. Ecological and economic relations of the crow, with special reference to Illinois. Ph.D. Thesis, University of Illinois at Urbana.

Brown, J. L. 1987. *Helping and Communal Breeding in Birds*. Princeton, New Jersey: Princeton University Press.

Buston, P. M., Reeve, H. K., Cant, M. A., Vehrencamp, S. L. & Emlen, S. T. 2007. Reproductive skew and the evolution of group dissolution tactics: a synthesis of concession and restraint models. *Animal Behaviour*, **74**, 1643-1654. 10.1016/j.anbehav.2007.03.003|ISSN 0003-3472.

Caffrey, C. 1992. Female biased delayed dispersal and helping in American crows. *The Auk*, **109**, 609-619.

Caffrey, C. 2000. Correlates of reproductive success in cooperatively breeding western American crows: if helpers help, it's not by much. *The Condor*, **102**, 333-341.

Cant, M. A. & Reeve, H. K. 2002. Female control of the distribution of paternity in cooperative breeders. *American Naturalist*, **160**, 602-611.

Chamberlain-Auger, J. A., Auger, P. J. & Strauss, E. G. 1990. Breeding biology of American crows. *Wilson Bulletin*, **102**, 615-622.

Clark, A. B., Robinson, D. A. & McGowan, K. J. 2006. Effects of West Nile virus mortality on social structure of an American crow (*Corvus brachyrhynchos*) population in New York state. *Ornithological Monographs*, **60**, 65-78.

Clutton-Brock, T. H. 1998. Reproductive skew, concessions and limited control. *Trends in Ecology & Evolution*, **13**, 288-292.

Double, M. & Cockburn, A. 2000. Pre-dawn infidelity: females control extrapair mating in superb fairy-wrens. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **267**, 465-470.

Eliassen, S. & Kokko, H. 2008. Current analyses do not resolve whether extrapair paternity is male or female driven. *Behavioral Ecology and Sociobiology*, **62**, 1795-1804.

Emlen, J. T., Jr. 1936. Age determination in the American crow. *Condor*, **38**, 99-102.
Emlen, S. T. 1996. Reproductive sharing in different types of kin associations. *American Naturalist*, **148**, 756-763.

Fridolfsson, A. K. & Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology*, **30**, 116-121.

Gowaty, P. A. & Buschhaus, N. 1998. Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis and social monogamy. *American Zoologist*, **38**, 207-225.

Hamilton, W. D. 1964. The genetical evolution of social behavior. *Journal of Theoretical Biology*, **7**, 1-52.

Heinsohn, R., Dunn, P., Legge, S. & Double, M. 2000. Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **267**, 243-249.

Johnstone, R. A. 2000. Models of reproductive skew: a review and synthesis. *Ethology*, **106**, 5-26.

Johnstone, R. A. & Cant, M. A. 1999. Reproductive skew and the threat of eviction: a new perspective. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **266**, 275-279.

Johnstone, R. A., Woodroffe, R., Cant, M. A. & Wright, J. 1999. Reproductive skew in multimember groups. *American Naturalist*, **153**, 315-331.

Kalinowski, S. T., Taper, M. L. & Marshall, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099-1106.

Kilham, L. 1984. Intra- and extrapair copulatory behavior in American Crows. *Wilson Bulletin*, 96.

Koenig, W. D. & Dickinson, J. L. 2004. *Ecology and Evolution of Cooperative Breeding in Birds*. Cambridge: Cambridge University Press.

Koenig, W. & Haydock, J. 2004. Incest avoidance. In: *Ecology and Evolution of Cooperative Breeding in Birds* (Ed. by W. D. Koenig & J. L. Dickinson), pp. 142-156. Cambridge: Cambridge University Press.

Koenig, W. D., Haydock, J. & Stanback, M. T. 1998. Reproductive roles in the cooperatively breeding acorn woodpecker: incest avoidance versus reproductive competition. *American Naturalist*, **151**, 243-255.

Kutsukake, N. & Nunn, C. L. 2006. Comparative tests of reproductive skew in male primates: the roles of demographic factors and incomplete control. *Behavioral Ecology and Sociobiology*, **60**, 695-706.

Langen, T. A. 1996. The mating system of the white-throated magpie-jay, *Calocitta formosa*, and Greenwood's hypothesis for sex biased dispersal. *Ibis*, **138**, 506-513.

Lawton, M. F. & Lawton, R. O. 1985. The breeding biology of the brown jay in Monteverde, Costa Rica. *Condor*, **87**, 192-204.

Li, S. H. & Brown, J. L. 2000. High frequency of extrapair fertilization in a plural breeding bird, the Mexican jay, revealed by DNA microsatellites. *Animal Behaviour*, **60**, 867-877.

Ligon, J. D. & Burt, D. B. 2004. Evolutionary origins. In: *Ecology and Evolution of Cooperatively Breeding in Birds* (Ed. by W. D. Koenig & J. L. Dickinson), pp. 5-34. Cambridge: Cambridge University Press.

Magrath, R. D. & Heinsohn, R. G. 2000. Reproductive skew in birds: models, problems and prospects. *Journal of Avian Biology*, **31**, 247-258.

Magrath, R. D., Johnstone, R. A. & Heinsohn, R. 2004. Reproductive skew. In: *Ecology and Evolution of Cooperatively Breeding in Birds* (Ed. by W. D. Koenig & J. L. Dickinson), pp. 157-176. Cambridge: Cambridge University Press.

Martin, P. & Bateson, P. 1993. *Measuring Behaviour*, 2nd. edn. Cambridge: Cambridge University Press.

Marzluff, J. M., McGowan, K. J., Donnelly, R. & Knight, R. L. 2001. Causes and consequences of expanding American Crow populations. In: *Avian Ecology and Conservation in an Urbanizing World* (Ed. by J. M. Marzluff, R. Bowman & R. Donnelly), pp. 365-381. Norwell, MA.: Kluwer Academic Press.

McGowan, K. J. 1995. A test of whether economy or nutrition determines fecal sac ingestion in nesting corvids. *Condor*, **97**, 50-56.

McGowan, K. J. 2001. Demographic and behavioral comparisons of suburban and rural American Crows. In: *Avian Ecology and Conservation in an Urbanizing World.* (Ed. by J. M. Marzluff, R. Bowman & R. Donelly), pp. 365-381. Norwell, MA.: Klumer Academic Press.

Nonacs, P. 2000. Measuring and using skew in the study of social behavior and evolution. *American Naturalist*, **156**, 577-589.

Nonacs, P. 2003. Measuring the reliability of skew indices: is there one best index? *Animal Behaviour*, **65**, 615-627.

Pradhan, G. R., Engelhardt, A., van Schaik, C. P. & Maestripieri, D. 2006. The evolution of female copulation calls in primates: a review and a new model. *Behavioral Ecology and Sociobiology*, **59**, 333-343. 10.1007/s00265-005-0075-y.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43**, 258-275.

Quinn, J. S., Woolfenden, G. E., Fitzpatrick, J. W. & White, B. N. 1999. Multi-locus DNA fingerprinting supports genetic monogamy in Florida Scrub-jays. Behavioral Ecology and Sociobiology, **45**, 1-10.

Rabenold, P. P., Rabenold, K. N., Piper, W. H., Haydock, J. & Zack, S. W. 1990. Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens. *Nature*, **348**, 538-540. doi:10.1038/348538a0.

Reeve, H. K. 2000. A transactional theory of within-group conflict. *American Naturalist*, **155**, 365-382.

Reeve, H. K. & Keller, L. 1996. Asymmetry and reproductive sharing in animal societies. *American Naturalist*, **148**, 764-769.

Reeve, H. K. & Keller, L. 2001. Tests of reproductive-skew models in social insects. *Annual Review of Entomology*, **46**, 347-385.

Reeve, H. K. & Shen, S. F. 2006. A missing model in reproductive skew theory: the bordered tug-of-war. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 8430-8434.

Reeve, H. K., Emlen, S. T. & Keller, L. 1998. Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? *Behavioral Ecology*, **9**, 267-278.

Schoenle, L. A., Townsend, A. K. & Lovette, I. J. 2007. Isolation and characterization of microsatellite loci in a cooperatively breeding corvid, the American crow (*Corvus brachyrhynchos*). *Molecular Ecology Notes*, **7**, 46-48.

Serrell, R. 2003. Sentinel behavior in the American crow *Corvus brachyrhynchos*. Master's thesis, Binghamton University.

Slate, J., Marshall, T. & Pemberton, J. 2000. A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Molecular Ecology*, **9**, 801-808.

Tarr, C. L. & Fleischer, R. C. 1998. Primers for polymorphic GT microsatellites isolated from the Mariana crow, *Corvus kubaryi*. *Molecular Ecology*, **7**, 253-255.

Vehrencamp, S. L. 1979. The roles of individual, kin and group selection in the evolution of sociality. In: *Handbook of Behavioral Neurobiology: Social Behavior and Communication* (Ed. by P. Marler & J. G. Vandenbergh), pp. 351-394. New York: Plenum Press.

Vehrencamp, S. L. 1983. A model for the evolution of despotic vs. egalitarian societies. *Animal Behaviour*, **31**, 667-682.

Verbeek, N. A. M. & Butler, R. W. 1999. Northwestern crow (*Corvus caurinus*). In: *The Birds of North America Online* (Ed. by A. Poole). Ithaca: Cornell Lab of Ornithology.

Verbeek, N. A. M. & Caffrey, C. 2002. American crow (*Corvus brachyrhynchos*). In: *The Birds of North America Online* (Ed. by A. Poole). Ithaca: Cornell Lab of Ornithology.

Westneat, D. F. & Stewart, I. R. K. 2003. Extrapair paternity in birds: causes, correlates and conflict. *Annual Review of Ecology Evolution and Systematics*, **34**, 365-396.

Westneat, D. F., Sherman, P. W. & Morton, M. L. 1990. The ecology and evolution of extrapair copulations in birds. In: *Current Ornithology* (Ed. by D. M. Power). New York & London: Plenum Press.

Wiley, R. H. & Rabenold, K. N. 1984. The evolution of cooperative breeding by delayed reciprocity and queuing for favorable social positions. *Evolution*, **38**, 609-621.

Williams, D. A. 2004. Female control of reproductive skew in cooperatively breeding brown jays (*Cyanocorax morio*). *Behavioral Ecology and Sociobiology*, **55**, 370-380.

Williams, D. A. & Rabenold, K. N. 2005. Male biased dispersal, female philopatry and routes to fitness in a social corvid. *Journal of Animal Ecology*, **74**, 150-159.

Wilson, T. M. 2008. Patterns of sentinel behavior at the nest in the cooperatively breeding American crow (*Corvus brachyrhynchos*), Master's Thesis, Binghamton University.

Woolfenden, G. E. & Fitzpatrick, J. W. 1984. *The Florida Scrub-jay: Demography of a Cooperative-Breeding Bird*. Princeton, NJ: Princeton University Press.

Yorzinski, J. L., Vehrencamp, S. L., Clark, A. B. & McGowan, K. J. 2006. The inflected alarm caw of the American crow: differences in acoustic structure among individuals and sexes. *Condor*, **108**, 518-529.

Young, A. J., Spong, G. & Clutton-Brock, T. 2007. Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 1603-1609.

CHAPTER 3

DISEASE-MEDIATED INBREEDING DEPRESSION IN A LARGE, OPEN POPULATION OF COOPERATIVE CROWS*

---

*A. K. Townsend., A. B. Clark, K. J. McGowan, E. L. Buckles, A. D. Miller, and I. J. Lovette. 2009. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proceedings of the Royal Society B. Reprinted with permission from the Royal Society.

Abstract

Disease-mediated inbreeding depression is a potential cost of living in groups with kin, but its general magnitude in wild populations is unclear. We examined the relationships between inbreeding, survival, and disease for 312 offspring, produced by 35 parental pairs, in a large, open population of cooperatively breeding American crows (*Corvus brachyrhynchos*). Genetic analyses of parentage, parental relatedness coefficients and pedigree information suggested that 23% of parental dyads were first- or second-order kin. Heterozygosity-heterozygosity correlations suggested that a microsatellite-based index of individual heterozygosity predicted individual genome-wide heterozygosity in this population. After excluding birds that died traumatically, survival probability was lower for more inbred birds during the 2-50 months after banding: the hazard rate for the most inbred birds was 170% higher than for the least inbred birds across the range of inbreeding index values. Birds that died with disease symptoms had higher inbreeding indices than birds with other fates. Our results suggest that avoidance of close inbreeding and the absence of inbreeding depression in large, open populations should not be assumed in taxa with kin-based social systems, and that microsatellite-based indices of individual heterozygosity can be an appropriate tool for examining inbreeding depression in populations where incest and close inbreeding occur.

Introduction

Relatively high pathogen exposure and transmission rates are a potential cost of group living (Alexander 1974; Schmid-Hempel & Crozier 1999) that might be elevated in taxa with kin-based social groups (Spottiswoode 2008), if related individuals share similar susceptibility characteristics (Shykoff & Schmid-Hempel 1991; Hughes &

Boomsma 2004) and/ or if pathogens are locally adapted to common genotypes (Lively et al. 2004). Furthermore, in the absence of active inbreeding avoidance mechanisms, taxa that live in kin groups (particularly those with limited natal dispersal of both sexes) might have a higher probability of mating with kin (Szulkin & Sheldon 2008), and offspring produced from these consanguineous matings might suffer even greater disease costs (Coltman et al. 1999). Inbred offspring have lower genome-wide heterozygosity than relatively outbred offspring, and might therefore experience a disease-mediated reduction in fitness if (1) they are unable to recognize as wide a breadth of pathogens as more heterozygous individuals (overdominance), and/ or (2) if pathogens are part of an environment that selects against individuals expressing deleterious recessive alleles (partial dominance) (Coltman et al. 1999). Disease-mediated inbreeding depression might therefore represent a substantial cost to living and breeding with kin, potentially influencing the evolution of dispersal and incest avoidance (Charlesworth & Charlesworth 1987). Understanding the relationship between inbreeding and disease is also important for the preservation of small, declining populations, in which inbreeding is unavoidable (Hedrick & Kalinowski 2000; Keller & Waller 2002).

Because empirical data on inbreeding depression in wild populations is limited (Keller & Waller 2002), particularly in terms of disease (Spielman et al. 2004), the general magnitude and frequency of disease-mediated inbreeding depression is unclear. In laboratory settings, some studies suggest that inbreeding increases susceptibility to pathogens or parasites (Luong et al. 2007; Ilmonen et al. 2008), whereas others have found that the relationship between disease resistance and inbreeding varies with the nature of the immune challenge (Calleri et al. 2006). Among Gila topminnows (*Poeciliopsis occidentalis*), for example, the relationship between fluke infection and inbreeding varied with source population (Hedrick et al.

2001), and relatively inbred individuals from all populations had higher survival after experimental infection with a novel bacterium (Giese & Hedrick 2003). Relatively low disease costs might be expected among habitual inbreeders, particularly under benign environmental conditions (Armbruster & Reed 2005), if long-term inbreeding purges the population's genetic load of deleterious alleles (Barrett & Charlesworth 1991), although potential overdominance might limit the success of purging (Crnokrak & Barrett 2002), and purging does not appear to operate consistently in wild populations (Byers & Waller 1999).

Inbreeding depression measured in the captive populations or laboratory settings might underestimate costs in wild populations (Crnokrak & Roff 1999). Because adequate pedigree information is often unavailable, most recent studies of inbreeding depression in wild populations have relied on microsatellite heterozygosity to infer genome-wide heterozygosity and inbreeding (Hansson & Westerberg 2002; Keller & Waller 2002; Coltman & Slate 2003). Some of these studies have found a positive association between microsatellite-based estimates of individual homozygosity (and potential inbreeding) and ectoparasite burden (Whiteman et al. 2006), endoparasite burden (Coltman et al. 1999; MacDougall-Shackleton et al. 2005; Acevedo-Whitehouse et al. 2006; Rijks et al. 2008), and mortality during epidemics (Valsecchi et al. 2004; Ross-Gillespie et al. 2007). Likewise, Acevedo-Whitehouse et al (2003) found an association between different diseases and marker-based estimates of individual heterozygosity in rehabilitated California sea lions (*Zalophus californianus)*. Other studies, however, have found no association between marker-based heterozygosity and endoparasites (Cote et al. 2005), and negative results might be underreported because of a publication bias towards significant correlations (Coltman & Slate 2003). It is difficult to assess the generality of disease-mediated inbreeding depression in natural populations with the available evidence.

The use of microsatellite markers to infer inbreeding coefficients could contribute to apparent variation in the relationship between disease and inbreeding among studies, because microsatellite heterozygosity is unlikely to predict the inbreeding coefficient in all systems (Hansson & Westerberg 2002; Balloux et al. 2004; Slate et al. 2004; DeWoody & DeWoody 2005). Microsatellite and genome-wide heterozygosity are expected to be most strongly correlated in very small populations with a high variance in inbreeding and a high proportion of incestuous matings, a scenario that is uncommon in nature (Balloux et al. 2004; Slate et al. 2004). If microsatellite and genome-wide heterozygosity are correlated in a given system, then heterozygosity estimated from one set of microsatellites should be positively correlated with heterozygosity from an independent set of microsatellites from the same individual ("heterozygosity-heterozygosity correlations" or HHCs; Balloux et al. 2004). Pedigree information and HHCs can be used together to examine (1) how well microsatellite and genome-wide heterozygosity are correlated in a given system, and (2) whether heterozygosity-fitness correlations are likely to be explained (at least in part) by inbreeding.

In this study, we examine disease-mediated inbreeding costs in American crows (*Corvus brachyrhynchos*), a species that occupies a wide range of habitats and is capable of long-distance migration (Verbeek & Caffrey 2002). American crows in a wild, cooperatively breeding population in Ithaca, New York, exhibit natal philopatry and limited natal dispersal of both sexes, as well as incest and inbreeding (Townsend et al. 2009). We first assessed the appropriateness of our inbreeding index, estimated from a panel of ten microsatellite markers, by comparing inbreeding indices with available pedigree information and parental relatedness coefficients, and through heterozygosity-heterozygosity correlations. We then explored the relationship between this inbreeding index and two indices of fitness: (1) survival within the duration of the

study (2-50 months after banding, depending on year in which an individual was sampled), and (2) the probability of dying with disease symptoms.

Methods

Field sampling

From 2004-2008, we collected blood via brachial venipuncture from 312 nestlings (Table 3.1) belonging to 30 American crow family groups in a long-term study population in Ithaca, New York (described in McGowan 2001; Townsend et al. 2009). Offspring were marked individually with color bands, aluminum bands, and patagial tags on day 23-30 after hatching. We collected genetic samples from blood or passively molted feathers from all members of the family groups of 283 of these 312 nestlings (Townsend et al. 2009). We monitored marked focal offspring for survival and poxviral dermatitis lesions at least once per month from their initial marking until July 2008.

Table 3.1. Number of offspring (*n*) marked in each year of the study and the maximum number of months that individuals from each cohort were monitored

Year	*N*	Months monitored
2004	35	50
2005	63	38
2006	73	26
2007	81	14
2008	60	2

(The number of months monitored represents a maximum because some individuals from each cohort died or disappeared before the endpoint of the study.)

Genetic analyses

We genotyped 312 nestlings and associated family members at ten microsatellite loci (Tarr & Fleischer 1998; Schoenle et al. 2006; Townsend et al. 2009). We assessed parentage of 283 nestlings with genotyped social parents using the maximum likelihood method in the program CERVUS 3.0 (Kalinowski et al. 2007), identifying probable genetic parents (within-pair and extra-pair) following criteria described in Townsend et al. (2009). For pairs identified as first-order kin by pedigree, we tested this degree of relatedness based on their genotypic information in the program KINGROUP ($R_p = 0$, $R_m = 1$; 100,000 simulations; $\alpha = 0.05$; Konovalov et al. 2004). We then assessed relatedness between the parental pair dyads for which we lacked pedigree information in KINGROUP, setting the selection criterion to identify pairs that were likely to be second-order kin ($R_p = 0$, $R_m = 0.5$). More markers (>17) would have been necessary to accurately assess deeper relationships (Goodnight & Queller 1999; Konovalov et al. 2004). We also used KINGROUP to estimate relatedness coefficients between all parental dyads. We estimated internal relatedness (IR), microsatellite-based inbreeding index that accounts for background allele frequencies when estimating parental similarity from an offspring's microsatellite genotype (Amos et al. 2001), using IRMacroN4 (http://www.zoo.cam.ac.uk/zoostaff/amos/#ComputerPrograms). To examine the relationship between IR and parental relatedness, we regressed offspring IR values against the KINGROUP-generated relatedness coefficients of their respective genetic parents in a mixed model with parental pair as a random factor.

We used a combination of pedigree information and HHCs to examine how well microsatellite and genome-wide heterozygosity were correlated within individuals in this system. First, we divided offspring into three groups: offspring produced incestuously, offspring produced through second-order kin matings, and

relatively outbred offspring. Following Balloux et al. (2004), we then generated HHCs for each of these three groups of offspring by 1) randomly splitting the ten loci into two sets of five independent loci, 2) calculating two IR values—one from each set of five loci—for each offspring, 3) regressing the two IR values against one another for all offspring in each group and calculating the r^2 value of the regressions, and 4) repeating this procedure 50 times. We then used analysis of variance to compare the 50 r^2 values generated for each of the three groups of offspring, predicting that mean r^2 value would be highest for the offspring produced incestuously and close to zero for the relatively outbred offspring (Balloux et al. 2004).

Fate determination

Dead crows were tested for West Nile virus (WNV) using reverse polymerase chain reaction (Clark et al. 2006). Birds that tested negative for WNV were necropsied with a complete external and internal examination. Dead crows discovered after November 2006 were subjected to gross examination and full necropsy, followed by sampling of all major organs with fixation in 10% neutral buffered formalin. Organs were sectioned using a tissue cutting knife, embedded in paraffin, microtome sectioned at 4 or 5 µm, and stained with hematoxylin and eosin using standard histological technique. Additional sections were also prepared for histochemical and immunohistochemical staining using the same protocol. All prepared sections were mounted with non-aqueous permanent mounting medium and analyzed under light microscopy by two veterinary anatomic pathologists.

IR and survival

To examine the relationship between IR and survival, we used Cox's proportional hazards regression and mark-recapture analyses. There was no evidence of

nonproportionality in the data. Year and sex had nonsignificant effects and were removed from the final proportional hazards regression model. Capture-history matrices were constructed using resight data from 171 individuals for which we had the most consistent resight data from the 2005-2007 cohorts during the first 14 months after banding, divided into 10 time intervals (May, June, July, August-December, January, February, March, April, May, June-July). Multiple resights within intervals were treated as a single sighting. Survival (Φ) and recapture (*p*) parameters were estimated in the program MARK 5.1 (http://www.phidot.org/software/mark/index.html). Following Lebreton et al. (1992), model selection was made using the Akaike Information Criterion (AIC; Akaike 1973). First, we generated models to detect time (t), year (y), and sex (s) effects on Φ and *p*, starting with [Φ(s*t + y*t)*p*(t)] as the global model. We estimated a quasi-likelihood parameter by dividing the deviance estimate from the original data by the mean of the simulated deviances from a parametric goodness of fit test (1000 bootstrap samples), adjusting the overdispersion parameter to 1.15. We then constrained the best model with inbreeding index as an individual covariate. The model with the lowest quasi AIC (QAIC) was accepted as the most parsimonious model for the data.

IR and fate

We explored the relationship between inbreeding index and fate in a mixed model with family as a random factor and fate as a fixed factor. Inbreeding index was normally distributed. Year and sex had nonsignificant effects and were removed from the final fate model. To examine the influence of the most inbred birds on heterozygosity-fitness correlations, we then excluded from fate and survival analyses

the 10 offspring (constituting 3% of the entire sample) that were known by pedigree to have been produced incestuously. Statistical analyses were conducted in JMP v 7.0.

Results

Genetic analyses

Thirty-five genetic parental pairs (including within-pair and extra-pair sires; Townsend et al. 2009) were identified for 230 offspring. Three of the 35 identified genetic parental pairs were first-order kin (mother-son extra-pair matings) by pedigree. KINGROUP identified these three genetic pairs as probable first-order kin, and identified five additional pairs as probable second-order kin, suggesting that 8 of the 35 identified genetic parental pairs (23%) were first- or second-order kin. First-order genetic pairs produced 10 of these 230 offspring (4.3%), whereas probable second-order genetic pairs produced 33 of the 230 offspring (14.3%). Mean relatedness coefficients between genetic parental pairs, estimated by KINGROUP, was 0.06 (range = -0.32 – 0.59). Individual IR of the 230 offspring with genotyped genetic parents was positively correlated with parental relatedness in a mixed model with parental pair as a random effect (0.54 ± 0.05 s.e., $F_{1,25.5} = 114.7$, $P < 0.0001$, $R^2 = 0.40$; Figure 3.1). There was significant variation in the strength of HHCs among offspring of different relatedness classes ($F_{2, 147} = 20.0$, $P < 0.001$, $n = 150$): the strength of the correlation, as expected, decreased as parental relatedness decreased (Tukey's HSD, $\alpha = 0.05$; Figure 3.2) and was close to zero for the relatively outbred birds.

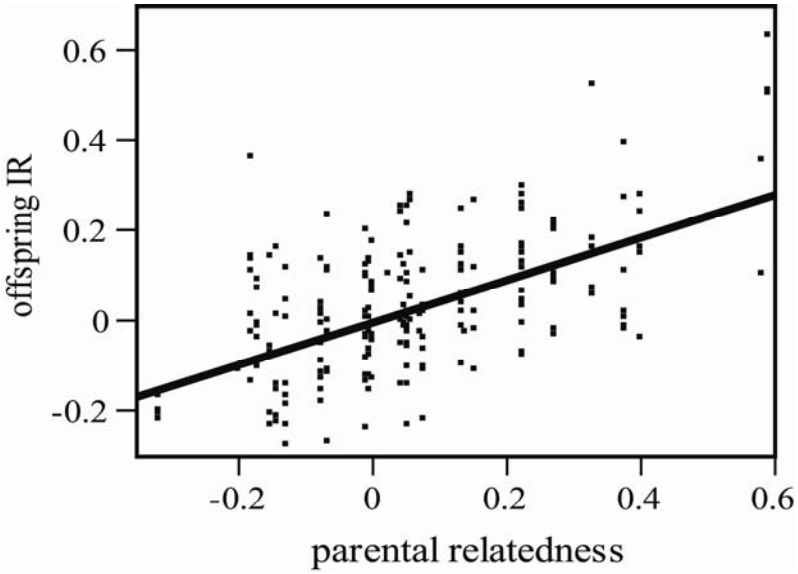

Figure 3.1. Relationship between offspring internal relatedness (IR) and parental relatedness coefficient. Ordinary least squares regression illustrated (0.53 ± 0.05 s.e., t_{228} =11.7, $p < 0.001$).

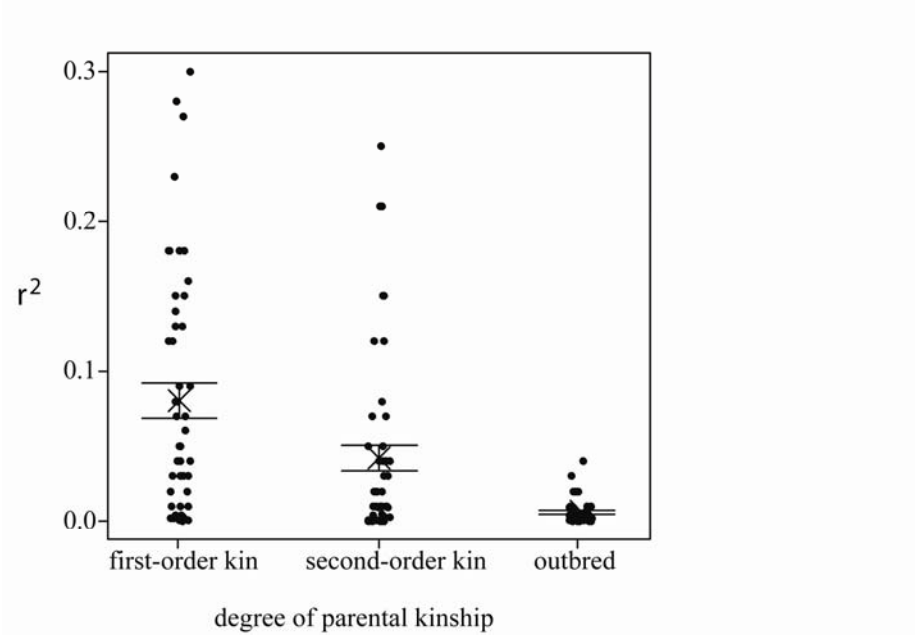

Figure 3.2. Heterozygosity-heterozygosity correlations (HHCs) for offspring produced by parents of different degrees of probable relatedness (e.g., first-order kin, second-order kin, and relatively outbred parental pairs). r^2 values in each group were obtained by randomly dividing the 10 loci into two groups of five loci, computing IR for both sets of loci for each offspring, regressing one against the other, and resampling the data 50 times. Means and standard errors shown.

Offspring fates

We placed the marked focal offspring into four fate categories: "alive" (n = 100) if they were seen within the last three months of the study; "trauma" (n = 67) if they died as a result of predation, car collisions, electrocution, shootings, and other violent deaths; "unknown" (n = 124) if they disappeared from the population, or if they were found dead but the cause of death was uncertain; and "diseased" (n = 21) if they had poxviral dermatitis lesions when they died or disappeared from the population (n = 14), if they tested positive for West Nile virus (WNV, n = 3), bacterial infections (n = 2), fungal pneumonia (n =1), or enteritis (n = 1).

IR, survival, and fate

Mark-recapture estimates of survival during the first year after banding was lower for offspring with higher inbreeding indices, but only after individuals that died traumatically (deaths that were potentially independent of individual condition) were removed from the analyses: there was considerable support for a time-dependent model with inbreeding index as an additive effect (Δ QAIC = 6.6; Table 3.2).

Table 3. 2. Candidate set of approximating models generated to fit American crow mark-recapture data

Model	$QAIC_c$	$\Delta QAIC_c$	$QAIC_c$ weight	*np*	Deviance
Φ(IR+t)*p*(t)	1882.413	0	0.963	19	1843.921
Φ(t)*p*(t)	1889.014	6.601	0.035	18	1852.571
Φ(t+s+y)*p*(t)	1894.886	12.4739	0.002	22	1850.231
Φ(t*y)*p*(t)	1909.346	26.9338	0	45	1816.623
Φ(t*s)*p*(t)	1919.354	36.9411	0	36	1845.611
Φ(t*s)+(t*y)*p*(t)	1921.579	39.1662	0	54	1809.648

np = number of parameters; Φ = survival; IR = internal relatedness; *p* = recapture; t = time; s = sex; y = year

Survival analyses also suggested that survival probability in the first 2-50 months after banding was lower for more inbred birds (Figure 3.3): the hazard rate for death or disappearance was 170% (95% CI: 2-564%) higher for the most inbred birds across the range of IR values (Table 3.3, model 1), again after excluding birds that died traumatically. Inbreeding index had an even stronger effect on survival when we considered only the birds in the "alive" and "diseased" categories, the fate categories most likely to be influenced by individual condition (Table 3.3, model 2).

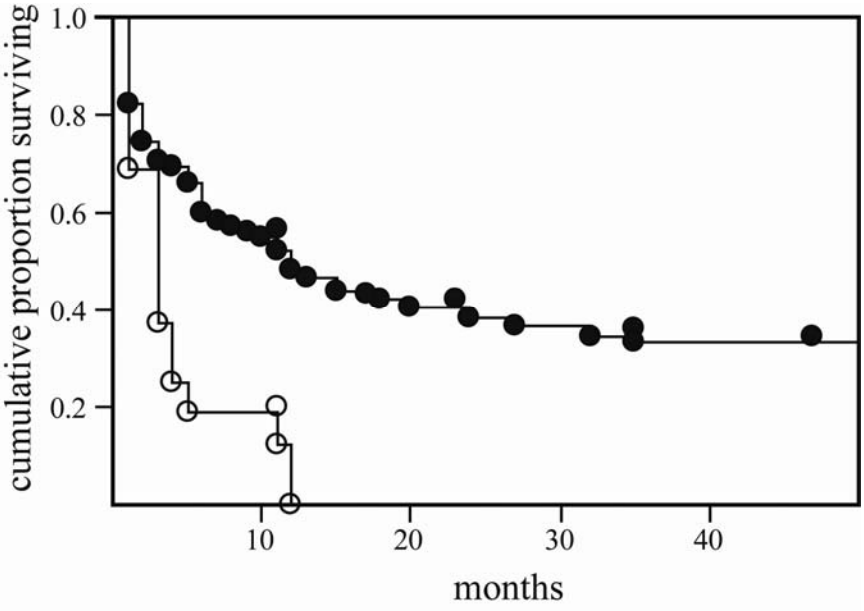

Figure 3.3. Survival with inbreeding index. Kaplan–Meier plot showing that highly inbred birds (IR > 0.35, n = 18, open markers) had lower proportional survival than relatively outbred birds (IR < 0.35, n = 294, closed markers; Log-rank: $X^2 = 8.37$, P = 0.004). Although presented categorically here for the purpose of illustration, inbreeding index was treated as a continuous variable in all other analyses.

Table 3.3. Internal relatedness (IR) and risk of death or disappearance of American crows. Model 1 includes crows that were alive, had died with disease symptoms, or were of unknown fate by the end of the study, whereas Model 2 includes just those that were alive or had died with disease symptoms by the end of the study.

model	n[a]	df	parameter estimate for IR	SE	X^2	*P*	risk ratio (RR)	95% confidence limits for RR	
1	245	1	1.0	0.47	4.0	0.047	2.70	1.02	6.64
2	121	1	4.8	1.0	19.1	<0.001	126.7	16.46	851.96

[a]Number of birds included in the analysis

IR varied with fate: in a mixed model with family as a random factor ($F_{3, 299.5} = 3.29$, $P = 0.02$), individuals that died with disease symptoms had significantly higher inbreeding indices than all other individuals (Tukey's HSD, $\alpha = 0.05$; Figure 3.4). It is possible that death from WNV was independent of individual quality, because in laboratory trials, all American crows died after WNV infection (Komar et al. 2003). Removing WNV-positive birds from the analysis did not change the association between inbreeding index and fate (mixed model with family as a random factor and fate as a fixed factor, $F_{3, 293.2} = 2.99$, $P = 0.03$, $n = 309$). When the 10 offspring that were known, by pedigree, to have been produced incestuously were removed from the sample, inbreeding index had no effect on nestling survival ($X^2 = 2.0$, $P = 0.16$, $n = 235$), and inbreeding index did not vary with fate ($F_{3, 290.9} = 2.3$, $P = 0.08$). Post-hoc tests for local and/ or direct effects (Hansson & Westerberg 2002), in which we reran the survival analysis and compared inbreeding index among the different fate categories with each locus sequentially removed (Hawley et al. 2005), yielded similar patterns, suggesting that these patterns were not driven by any single locus.

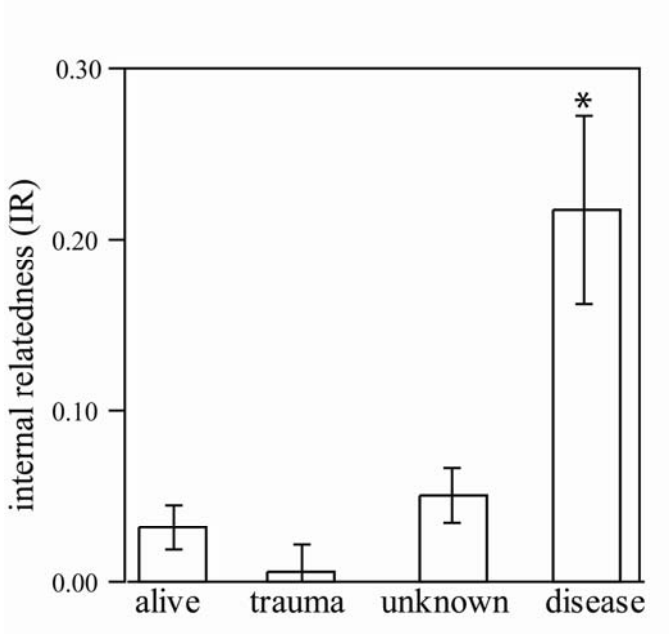

Figure 3.4. Internal relatedness (IR) of offspring in different fate classes. Means and standard errors shown.

Discussion

We have shown evidence for a substantial survival cost of inbreeding in this vast, contiguous and open crow population. Survival in the 2-50 months after banding was lower for more inbred birds: the hazard rate for the most inbred birds was 170% higher than for the least inbred birds across the range of inbreeding index values, after we excluded birds that died traumatically from the sample. Reduced survival for inbred birds appeared to be mediated, at least in part, by disease: birds that died with disease symptoms had higher inbreeding indices than those lived for the duration of the study, died traumatically, or those whose cause of death or disappearance was ambiguous. This disease-mediated inbreeding depression represents the minimum cost of inbreeding in this population, because apparent costs tend to accumulate with life stages and fitness indices measured (Pusey & Wolf 1996). We did not account for

potential inbreeding depression expressed early in development, if inbred individuals were more likely to die embryonically or soon after hatching (Keller & Waller 2002) or later in life, if inbred individuals had a lower probability of survival in later life stages, were less fecund (Spottiswoode & Moller 2004), or were less successful in acquiring mates (Pusey & Wolf 1996; Seddon et al. 2004; Hoffman et al. 2007).

Despite the high frequency of reported microsatellite-based heterozygosity-fitness correlations (Coltman & Slate 2003), a number of authors have suggested that microsatellite-based estimates of heterozygosity might generally be of limited use as indices of inbreeding (Balloux et al. 2004; Slate et al. 2004; DeWoody & DeWoody 2005). Our results suggest that they can be useful in populations where incestuous matings and matings between second-order kin occur. Heterozygosity-heterozygosity correlations, as well as the positive correlation between parental relatedness coefficients and offspring IR, suggested that our microsatellite-based estimates of individual heterozygosity did indeed reflect inbreeding in this population. The relatively strong correlation between IR and genome-wide heterozygosity for the most inbred birds was not surprising, given that recent inbreeding events are expected to have a much larger effect on inbreeding coefficient than inbreeding events deeper in the pedigree (Balloux et al. 2004).

Kin matings in this population might occur incidentally from living in close proximity to sexually mature, opposite-sex kin. In order for limited natal dispersal of both sexes to have persisted in this population, we might expect that inbreeding costs are balanced by the benefits gained from living and/ or breeding with kin (Alexander 1974). Various potential benefits have been proposed for living with kin, such as enhanced fitness of non-descendent kin (Emlen 1995; but see Caffrey 2000), nepotistic defence (Sherman 1981), enhanced survival (Ekman et al. 2000), lineage persistence (Marzluff & Balda 1990), and territorial inheritance (Woolfenden &

Fitzpatrick 1978). Potential benefits of kin matings themselves include kin selection, which can, in theory, outweigh surprisingly high inbreeding depression costs (Kokko & Ots 2006), and the maintenance of locally selected gene complexes, which could be disrupted through matings with individuals from other populations ("outbreeding depression;" Shields 1982; Bateson 1983). In this American crow population, individuals exhibit habitat specificity, tending to breed in microhabitats (urban or rural) similar to their natal territory (McGowan 2001). Matings between birds adapted to the same microhabitat (such as kin) might promote offspring adaptation to a particular microhabitat.

In order to avoid “too much” outbreeding, Bateson (1983) suggested that optimal mates might be those that are moderately related, particularly if any costs of mating with kin decline quickly with degree of parental relatedness. Disease-mediated costs of inbreeding might, indeed, have declined quickly with parental relatedness in this population: when we removed from our sample a small number of offspring that were known by pedigree to have been produced incestuously, significant patterns of survival and fate with IR disappeared. An alternative explanation for this result, however, is that we were only able to detect inbreeding depression in the most inbred offspring because our marker-based estimate of heterozygosity did not correlate well with genome-wide heterozygosity for relatively outbred offspring. Without additional molecular markers and pedigree information, we cannot determine whether relatively outbred birds did not suffer disease-mediated inbreeding costs or whether we were unable to detect these costs with our available marker set.

The results of this study are important for three reasons. First, although it often appears true that, when possible, incest and close inbreeding are avoided in cooperative breeders, particularly among cooperatively breeding birds (Koenig & Haydock 2004), our analyses suggest that close inbreeding is not uncommon in this

open population of crows (Townsend et al. 2009; this study). It is possible that incest and close inbreeding occurs undetected in other taxa because of the expectation that it will not occur (Kokko & Ots 2006), or because limited marker sensitivity makes the detection of incest in taxa that live in kin groups challenging (McRae & Amos 1999). Second, the severity of disease-mediated inbreeding costs that we detected was surprising, given that close inbreeding, if strongly selected against, could presumably have been avoided in this large, open population. Third, we found evidence for a correlation between marker-based estimates of heterozygosity and actual inbreeding in this population. Simulations by Balloux et al. (2004) suggested that microsatellite heterozygosity would be most likely to reflect genome-wide heterozygosity in populations where there is a high proportion of consanguineous matings, such as might be found in very small or subdivided populations, or those with highly skewed mating systems. In our sample, even though 23% of genetic pairs appeared to be first- or second-order kin, the proportion of offspring produced by these pairs was not high: only 4.3% of offspring were produced by first-order kin dyads, and 14.3% of offspring were produced by second-order kin dyads. Nevertheless, IR values estimated from our panel of ten microsatellite markers appeared sufficient to reflect differences in genome-wide heterozygosity between these highly inbred offspring and relatively outbred offspring. In conclusion, microsatellite markers, when verified as an actual index of inbreeding by even very shallow pedigree information, can serve as a valuable tool for quantifying inbreeding and inbreeding depression in populations in which some level of incest and close inbreeding occurs. Care must be taken to evaluate the assumption of incest avoidance in each system, as well as the assumption that inbreeding costs will be low in large, open populations, particularly for taxa that live in kin groups.

REFERENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D. & Amos, W. 2003 Disease susceptibility in California sea lions. *Nature* **422**, 35.

Acevedo-Whitehouse, K., Spraker, T. R., Lyons, E., Melin, S. R., Gulland, F., Delong, R. L. & Amos, W. 2006 Contrasting effects of heterozygosity on survival and hookworm resistance in California sea lion pups. *Mol. Ecol.* **15**, 1973-1982.

Akaike, H. 1973 Information theory as an extension of the maximum likelihood principle. In *Second International Symposium on Information Theory* (ed. B. N. Petrov & F. Csaki), pp. 267-281. Budapest: Akademiai Kiado.

Alexander, R. D. 1974 The evolution of social behavior. *Annu. Rev. Ecol. Syst.* **5**, 325-383.

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001 The influence of parental relatedness on reproductive success. *Proc. R. Soc. B* **268**, 2021-2027.

Armbruster, P. & Reed, D. H. 2005 Inbreeding depression in benign and stressful environments. *Heredity* **95**, 235-242.

Balloux, F., Amos, W. & Coulson, T. 2004 Does heterozygosity estimate inbreeding in real populations? *Mol. Ecol.* **13**, 3021-3031.

Barrett, S. C. H. & Charlesworth, D. 1991 Effects of a change in the level of inbreeding on the genetic load. *Nature* **352**, 522-524.

Bateson, P. 1983 Optimal outbreeding. In *Mate Choice* (ed. P. Bateson), pp. 257-277. Cambridge University Press.

Byers, D. L. & Waller, D. M. 1999 Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Sys.* **30**, 479-513.

Caffrey, C. 2000 Correlates of reproductive success in cooperatively breeding western American crows: if helpers help, it's not by much. *Condor* **102**, 333-341.

Calleri, D. V., Reid, E. M., Rosengaus, R. B., Vargo, E. L. & Traniello, J. F. A. 2006 Inbreeding and disease resistance in a social insect: effects of heterozygosity on immunocompetence in the termite *Zootermopsis angusticollis*. *Proc. R. Soc. B* **273**, 2633-2640.

Charlesworth, D. & Charlesworth, B. 1987 Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Sys.* **18**, 237-268.

Clark, A. B., Robinson, D. A. & McGowan, K. J. 2006 Effects of West Nile virus mortality on social structure of an American crow (*Corvus brachyrhynchos*) population in New York state. *Ornithol. Monogr.* **60**, 65-78.

Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J. M. 1999 Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* **53**, 1259-1267.

Coltman, D. W. & Slate, J. 2003 Microsatellite measures of inbreeding: A meta-analysis. *Evolution* **57**, 971-983.

Cote, S. D., Stien, A., Irvine, R. J., Dallas, J. F., Marshall, F., Halvorsen, O., Langvatn, R. & Albon, S. D. 2005 Resistance to abomasal nematodes and individual genetic variability in reindeer. *Mol. Ecol.* **14**, 4159-4168.

Crnokrak, P. & Barrett, S. C. H. 2002 Purging the genetic load: A review of the experimental evidence. *Evolution* **56**, 2347-2358.

Crnokrak, P. & Roff, D. A. 1999 Inbreeding depression in the wild. *Heredity* **83**, 260-270.

DeWoody, Y. D. & DeWoody, J. A. 2005 On the estimation of genome-wide heterozygosity using molecular markers. *J. Hered.* **96**, 85-88.

Ekman, J., Bylin, A. & Tegelstrom, H. 2000 Parental nepotism enhances survival of retained offspring in the Siberian jay. *Behav. Ecol.* **11**, 416-420.

Emlen, S. T. 1995 An evolutionary theory of the family. *Proc. Natl. Acad. Sci.* **92**, 8092-8099.

Giese, A. R. & Hedrick, P. W. 2003 Genetic variation and resistance to a bacterial infection in the endangered Gila topminnow. *Anim. Conserv.* **6**, 369-377.

Goodnight, K. F. & Queller, D. C. 1999 Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol.* **8**, 1231-1234.

Hansson, B. & Westerberg, L. 2002 On the correlation between heterozygosity and fitness in natural populations. *Mol. Ecol.* **11**, 2467-2474.

Hawley, D. M., Sydenstricker, K. V., Kollias, G. V. & Dhondt, A. A. 2005 Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. *Biol. Letters* **1**, 326-329.

Hedrick, P. W. & Kalinowski, S. T. 2000 Inbreeding depression in conservation biology. *Annu. Rev. Ecol. Sys.* **31**, 139-162.

Hedrick, P. W., Kim, T. J. & Parker, K. M. 2001 Parasite resistance and genetic variation in the endangered Gila topminnow. *Anim. Conserv.* **4**, 103-109.

Hoffman, J. I., Forcada, J., Trathan, P. N. & Amos, W. 2007 Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* **445**, 912-914.

Hughes, W. O. H. & Boomsma, J. J. 2004 Genetic diversity and disease resistance in leaf-cutting ant societies. *Evolution* **58**, 1251-1260.

Ilmonen, P., Penn, D. J., Damjanovich, K., Clarke, J., Lamborn, D., Morrison, L., Ghotbi, L. & Potts, W. K. 2008 Experimental infection magnifies inbreeding depression in house mice. *J. Evol. Biol.* **21**, 834-841.

Kalinowski, S. T., Taper, M. L. & Marshall, T. C. 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16, 1099-1106.

Keller, L. F. & Waller, D. M. 2002 Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230-241.

Koenig, W. & Haydock, J. 2004 Incest avoidance. In *Ecology and Evolution of Cooperative Breeding in Birds* (ed. W. Koenig & J. Dickinson), pp. 142-156, Cambridge University Press.

Kokko, H. & Ots, I. 2006 When not to avoid inbreeding. *Evolution* **60**, 467-475.

Komar, N., Langevin, S., Hinten, S., Nemeth, N., Edwards, E., Hettler, D., Davis, B., Bowen, R. & Bunning, M. 2003 Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg. Infect. Dis.* **9**, 311-322.

Konovalov, D. A., Manning, C. & Henshaw, M. T. 2004 KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Mol. Ecol. Notes* **4**, 779-782.

Lebreton, J. D., Burnham, K. P., Clobert, J. & Anderson, D. R. 1992 Modeling survival and testing biological hypotheses using marked animals - a unified approach with case studies. *Ecol. Monogr.* **62**, 67-118.

Lively, C. M., Dybdahl, M. F., Jokela, J., Osnas, E. E. & Delph, L. F. 2004 Host sex and local adaptation by parasites in a snail-trematode interaction. *Am. Nat.* **164**, S6-S18.

Luong, L. T., Heath, B. D. & Polak, M. 2007 Host inbreeding increases susceptibility to ectoparasitism. *J. Evol. Biol.* **20**, 79-86.

MacDougall-Shackleton, E. A., Derryberry, E. P., Foufopoulos, J., Dobson, A. P. & Hahn, T. P. 2005 Parasite-mediated heterozygote advantage in an outbred songbird population. *Biol. Letters* **1**, 105-107.

Marzluff, J. M. & Balda, R. P. 1990 Pinyon Jays: making the best of a bad situation by helping. In *Cooperative Breeding in Birds* (ed. P. Stacey & W. Koenig), pp. 197-238. Cambridge University Press.

McGowan, K. J. 2001 Demographic and behavioral comparisons of suburban and rural American Crows. In *Avian Ecology and Conservation in an Urbanizing World* (ed. J. Marzluff, R. Bowman & D. Donelly), pp. 365-381. Norwell, MA.: Klumer Academic Press.

McRae, S. B. & Amos, W. 1999 Can incest within cooperative breeding groups be detected using DNA fingerprinting? *Behav. Ecol. Sociobiol.* **47**, 104-107.

Pusey, A. & Wolf, M. 1996 Inbreeding avoidance in animals. *Trends Ecol. Evol.* **11**, 201-206.

Rijks, J. M., Hoffman, J. I., Kuiken, T., Osterhaus, A. & Amos, W. 2008 Heterozygosity and lungworm burden in harbour seals (*Phoca vitulina*). *Heredity* **100**, 587-593.

Ross-Gillespie, A., O'Riain, M. J. & Keller, L. F. 2007 Viral epizootic reveals inbreeding depression in a habitually inbreeding mammal. *Evolution* **61**, 2268-2273.

Schmid-Hempel, P. & Crozier, R. H. 1999 Polyandry versus polygyny versus parasites. *Philos. T. Roy. Soc. B* **354**, 507-515.

Schoenle, L. A., Townsend, A. K. & Lovette, I. J. 2007 Isolation and characterization of microsatellite loci in a cooperatively-breeding corvid, the American crow (*Corvus brachyrhynchos*). *Mol. Ecol. Notes* **7**, 46-48.

Seddon, N., Amos, W., Mulder, R. A. & Tobias, J. A. 2004 Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. *Proc. R. Soc. B* **271**, 1823-1829.

Sherman, P. W. 1981 Kinship, demography, and belding ground-squirrel nepotism. *Behav. Ecol. Sociobiol.* **8**, 251-259.

Shields, W. M. 1982 *Philopatry, Inbreeding, and the Evolution of Sex,* Albany:

State University of New York Press.

Shykoff, J. A. & Schmid-Hempel, P. 1991 Parasites and the advantage of genetic variability within social insect colonies. *Proc. R. Soc. B* **243**, 55-58.

Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E. & McEwan, J. C. 2004 Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **93**, 255-265.

Spielman, D., Brook, B. W., Briscoe, D. A. & Frankham, R. 2004 Does inbreeding and loss of genetic diversity decrease disease resistance? *Conserv. Genet.* **5**, 439-448.

Spottiswoode, C. & Moller, A. P. 2004 Genetic similarity and hatching success in birds. *Proc. R. Soc. B* **271**, 267-272.

Spottiswoode, C. N. 2008 Cooperative breeding and immunity: a comparative study of PHA response in African birds. *Behav. Ecol. Sociobiol.* **62**, 963-974.

Szulkin, M. & Sheldon, B. C. 2008 Dispersal as a means of inbreeding avoidance in a wild bird population. *Proc. R. Soc. B* **275**, 703-711.

Tarr, C. L. & Fleischer, R. C. 1998 Primers for polymorphic GT microsatellites isolated from the Mariana crow, *Corvus kubaryi*. *Mol. Ecol.* **7**, 253-255.

Townsend, A. K., Clark, A. B., McGowan, K. J. & Lovette, I. J. 2009 Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crow. *Anim. Behav.***77**, 503-512.

Valsecchi, E., Amos, W., Raga, J. A., Podesta, M. & Sherwin, W. 2004 The effects of inbreeding on mortality during a morbillivirus outbreak in the Mediterranean striped dolphin (*Stenella coeruleoalba*). *Anim. Conserv.* **7**, 139-146.

Verbeek, N. A. M. & Caffrey, C. 2002 The American Crow (*Corvus brachyrhynchos*). *Birds of North America* **647**.

Whiteman, N. K., Matson, K. D., Bollmer, J. L. & Parker, P. G. 2006 Disease ecology in the Galapagos Hawk (*Buteo galapagoensis*): host genetic diversity, parasite load and natural antibodies. *Proc. R. Soc. B* **273**, 797-804.

Woolfenden, G. E. & Fitzpatrick, J. W. 1978 Inheritance of territory in group-breeding birds. *Bioscience* **28**, 104-108.

CHAPTER 4

EXTRAPAIR COPULATIONS PREDICT EXTRAPAIR FERTILIZATIONS IN THE AMERICAN CROW*

---

*A. K. Townsend, 2009. Extrapair copulations predict extrapair fertilizations in the American Crow. The Condor 11:387-392.

Abstract

The general relationship between extrapair copulations (EPC) and extrapair paternity (EPP) in wild birds is unclear because relatively few studies have collected both types of information in a single population. I compared observed copulatory behavior with genetic paternity in a population of American Crows (*Corvus brachyrhynchos).* The proportion of extrapair young in a brood was higher when EPC attempts were observed in that group-year. Overall proportion of broods with extrapair young was identical to the proportion of focal group-years in which EPC attempts were observed (32%). In a given brood, however, observed EPC attempts did not always predict EPP, and failure to observe EPCs did not always predict monogamy. Furthermore, males observed attempting EPCs often differed from the males gaining EPP, suggesting that EPCs were attempted by multiple males with certain females in certain years. Observed EPC attempts were initiated by males, and most appeared to be resisted by females.

Introduction

Few socially monogamous species of birds are genetically monogamous (Griffith et al. 2002). For males, extrapair paternity (EPP) provides an opportunity to sire offspring outside of their social pair bonds, for which they usually provide little parental care. For females, EPP might provide an opportunity to acquire genetic or direct benefits from extrapair males (Jennions and Petrie 2000, Griffith et al. 2002), and the majority of current research has focused on the potential adaptive benefits of EPP for females (Griffith et al. 2002). Although evidence suggesting that females might seek their extrapair partners has been documented in a some species (Neudorf et al. 1997, Double and Cockburn 2000, Pedersen et al. 2006), relatively few extrapair copulations (EPCs) have actually been documented, and some of these appear to be unsolicited, or

even resisted, by females (reviewed in Westneat and Stewart 2003). Across taxa, evidence for genetic benefits of EPP derived by females is mixed (Akcay and Roughgarden 2007, Kempenaers 2007, Mays et al. 2008), and the degree to which we might expect EPP to be generally sought by females for its fitness benefits is debatable (Arnqvist and Kirkpatrick 2005, 2007, Griffith 2007). Available data appear insufficient to resolve the issue (Eliassen and Kokko 2008).

If EPP is generally male-driven in a given system, extrapair fertilizations could occur in two ways. First, extrapair males might coerce unwilling females to copulate, although the ability of males without intromittant organs to forcibly copulate is controversial (Gowaty and Buschhaus 1998, Westneat and Stewart 2003). Alternatively, females might submit to EPCs with some extrapair males to reduce the cost of harassment (Westneat and Stewart 2003, Arnqvist and Kirkpatrick 2007). In general, females would be expected to submit to EPCs when the costs of resisting exceed the costs of submitting (Eliassen and Kokko 2008). Even if some males do successfully copulate with unwilling females, however, females might be able to exercise post-copulatory choice over the success of the fertilization (Birkhead and Moller 1992; Gowaty and Buschhaus 1998). For example, given the potential costs of inbreeding depression (Keller and Waller 2002, Townsend et al 2009a), a female that is coerced into copulation with a closely related male might attempt to decrease the probability of successful fertilization (e.g., through sperm ejection; Birkhead and Moller 1992, page 82). Males are, however, likely to coevolve mechanisms to increase their probability of successful fertilizations (Westneat and Stewart 2003).

Many recent studies of the evolution of EPCs have focused entirely on genetic patterns of EPP, without corresponding information of how this paternity relates to copulatory behavior itself (Griffith 2007), and without information on whether males or females generally appear to seek EPCs in a given population (Westneat and Stewart

2003). Interspecific reviews have suggested that the frequency of observed EPC might have little or no predictive power about the level of EPP, and that the relationship between the two is indirect (Dunn and Lifjeld 1994, Birkhead and Moller 1995). Griffith (2007) suggested that the nature of the relationship between EPC and EPP is critical to understanding their functions, and that the following questions should be addressed in empirical populations: What is the proportion of females in a given population involved in EPCs? How does this proportion relate to the proportion of broods with EPP? What is the variation in extrapair behavior among females, and how does this relate to actual EPP?

I examined the relationship between observed EPC attempts and realized EPP in the cooperatively breeding American Crow (*Corvus brachyrhynchos*). Within-pair and extrapair copulatory behaviors have been described in New York and Florida populations (Kilham 1984, Townsend et al. 2009b). Although some of the American Crows in the New York population that attempted EPCs acquired paternity (Townsend et al. 2009b), the general relationship between observed EPC attempts and the occurrence of EPP in these crows is unclear. Inbreeding depression costs are high in the New York population: offspring produced by first- and second-order kin pairs have a lower survival probability and higher disease probability than relatively outbred offspring (Townsend et al. 2009a). Here, I address the following four questions: 1) Do observations of EPC attempts predict the proportion of extrapair young in a given brood? 2) Does the proportion of group-years in which EPC attempts are observed correspond to the proportion of broods containing extrapair young in the population? 3) Are the individual males observed attempting EPCs always the same males that acquire paternity? 4) Does relatedness of these prospective extrapair males to the female breeder influence their probability of acquiring paternity?

Methods

Study area and field observation

During 2005–2008, I examined mating behavior and genetic parentage in a suburban population of American Crows in Ithaca, New York, U.S.A. which has been monitored continuously since 1989 (McGowan 2001, Clark et al. 2006). Family groups in this population are characterized by a behaviorally distinct, socially monogamous pair, assisted at the nest by 0-10 auxiliaries of either sex (mean group size from 2004-2007 = 4± 0.22 birds, range 2–9 birds; Townsend et al. 2009b). Although these auxiliaries are often adult or subadult offspring from previous broods, some contain adult males that are unrelated to the female breeders (stepsons, non-descendent kin of the male breeder, or completely unrelated birds; described in Townsend et al. 2009b). The majority of birds belonging to focal family groups were banded or identifiable by unique scars. During most breeding attempts, there was not more than one unmarked individual in each group (range 0–2).

I monitored groups during February–July (2–7 days per week) to document group membership and social role, mate guarding, within-pair and extrapair copulations, incubation, hatching and fledging. During August–January, I observed each group at least once per month to record membership and interactions among members. In 2007-2008, I conducted focal observations on family groups during the nest-building, egg-laying and early incubation periods (ending observations by the second day of incubation). I recorded all within-pair and extrapair copulation attempts during one to four focal observations per family group (25–150 min per focal observation, depending on how long a family group could be followed on a given day). All members of focal families were tightly associated with the female breeders on their respective territories in this early breeding period. Group activities were conspicuously centered around prospective nest trees in open habitat (e.g., yards,

cemeteries, and golf courses), and therefore were easy to monitor. Observation periods on a given day began when the breeding female of a group was first located. Observation periods ended either after a predetermined period of time, or sooner, if the female of the group (generally closely accompanied by her pair male and other group members) disappeared from my line of sight behind a structural component on her territory (e.g., houses) or if the group flew out of my sight (usually as a cohesive unit) while chasing aerial predators or conspecific intruders. Females were therefore visible throughout the duration of the focal observations.

Genetic sampling and analyses

On days 24–30 after hatching, my collaborators climbed to each nest to mark nestlings with unique combinations of metal bands, color bands and patagial tags. We collected blood (~150 ul) from the brachial vein of live nestlings, and tissue samples from dead nestlings in and under these nests. I extracted DNA from 124 of the 125 marked and unmarked adult birds in these groups, using blood sampled from these individuals as nestlings, or passively molted feathers collected while the adults were provisioning nestlings or fledglings on their territories (June–August). Unmarked birds present in multiple years were regenotyped using new feathers collected each year to reconfirm their identity. One auxiliary that was present only in a single year disappeared before it could be sampled. I extracted DNA from blood samples using Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY, U.S.A.) and from feather tips using DNeasy tissue kits (Qiagen, Valencia, CA, U.S.A.). I genotyped offspring and family members at 10 polymorphic microsatellite loci (Tarr and Fleischer 1998, Schoenle et al. 2007), and I used the maximum likelihood approach for parentage analyses in the program CERVUS 3.0 (Kalinowski 2007), following Townsend et al. (2009b). Relatedness among group members and between breeding pairs was assessed using genetic and

pedigree data, following methods described in Townsend et al (2009a). I used the program KINGROUP (Konovalov et al. 2004) to estimate relatedness coefficients between all dyads of female with their pair males and with their prospective extrapair sires.

Statistical analyses

To examine the relationship between observed EPC attempts and EPP, I analyzed the proportion of offspring produced by extrapair males in a given brood as a function of observed EPC attempts (yes/no) in a generalized linear model (GLM) with binomial errors and logit link function, weighted by the total number of offspring in the brood, in R v.2.7.2 (R Development Core Team, 2008), using the broods for which I had corresponding focal observations in that group-year (n = 25 broods). There was no evidence of overdispersion in the data. One assumption of this model was that my likelihood of observing EPCs was not dependent on the time I spent watching a given group. This assumption appeared to be valid: in a one-tailed, two-sample t-test, the mean number of observation minutes for group-years in which I observed EPCs (226.6 ± 38.5 minutes, n = 8 group-years) was not significantly higher than the mean number of observation minutes for the other group-years (194.5 ± 31.4 minutes, n = 17 groups-years; $t(23) = -0.6$; $p = 0.28$). The values reported are means ± SE.

To examine whether probability of acquiring paternity varied with relatedness of prospective extrapair males to the female breeder, I analyzed acquisition of paternity (yes/no) of a given prospective extrapair sire as the response in a generalized linear mixed model (GLMM), with relatedness coefficients between dyads of prospective extrapair sires and females as the predictor. I specified binomial errors and family as a random effect, and used the Penalized Quasi-Likelihood method. I included in this analysis all genotyped males that were known to have attempted

EPCs, including both those that were observed attempting EPCs (n = 9 males; Table 4.1) and those that acquired EPP but were not observed attempting EPCs (n = 7 males). Similarly, to see if a prospective extrapair male was more likely to acquire paternity when he was less related to a female than she was to her social mate, I examined acquisition of paternity (yes/no) as the response in a GLMM with the difference between relatedness coefficients (i.e., the coefficient of the social pair minus the coefficient of the female-extrapair male dyad) as the predictor, again specifying binomial errors and family as a random effect.

Table 4.1. Observed EPC attempts, occurrence of EPP, and number and identity of males observed attempting EPCs. The male's relationship to the breeding female (when known) indicated by superscripts. Additional details provided in text.

Group	Number of auxiliaries observed attempting EPCs	Number of auxiliaries attaining EPP	EPCs by extragroup males observed?	EPP by extragroup males?
1	1[a]	1	No	No
2	1[b]	1	No	No
3	3[a]	1	No	No
4	1[b]	1[c]	No	No
5	1[c]	0	No	Yes
6	0	1[c]	Yes	No
7	1[b]	0	Yes	No
8	0	0	Yes	Yes
9	0	0	Yes	No

[a]Stepson; unrelated to the breeding female.
[b]Nondescendent kin of the breeding male; unrelated to the breeding female.
[c]Sons; first-order kin of the breeding female.

Results

I analyzed genetic paternity data in 71 broods from 25 family groups: 18 broods from 2005, 21 from 2006, 19 from 2007, and 13 from 2008. The number of analyzed broods varied annually because some broods failed before offspring were sampled. For 25 of these broods, I collected corresponding focal observations of copulatory activity in

their respective family between 17 March and 7 April 2007 and between 14 March and 5 April 2008. I conducted 85 focal observations for a total of 99.6 hours (mean number of observation minutes = 70.31 ± 3.13 per focal observation), with a mean of 2.65 ± 0.2 focal observations per group-year. During the other 46 groups-years, copulatory behavior was observed and recorded incidentally during nest-building, egg-laying and early incubation periods.

In the course of both the focal observations and incidental observations, I observed six within-pair copulations and 15 EPC attempts. Details of all within-pair copulations and 14 of these EPC attempts are given in Townsend et al (2009b). Prior to each within-pair copulation, the male breeder approached the female from the front and she performed precopulatory displays (described in Kilham 1984) lowering herself to the ground and quivering her wings. All within-pair copulations appeared successful. In contrast, all of the extrapair copulation attempts observed between 2005-2007 appeared to be unsolicited by, and vigorously resisted by, female breeders: females did not perform precopulatory displays prior to these copulation attempts and appeared to actively resist them, flapping their wings throughout and vocalizing loudly. All attempted EPCs were interrupted by the male breeder and none appeared to be successfully completed. An additional extrapair copulation attempt was observed in 2008. This EPC attempt was similar to the previous observations that were described in Townsend et al. (2009b) in that it appeared to be unsolicited by the female breeder, was not prefaced with precopulatory displays, and was quickly interrupted by the male breeder. It differed from previous observations of EPC attempts in that the female did not appear to resist: she lowered herself to the ground and did not appear to struggle. I did not observe females from other territories enter my focal territories and/or solicit extrapair copulations from any of my focal males.

Attempted EPCs were observed in 9 of the 25 family groups (36%), in 9 of 71 of group-years (13%). Most EPC attempts were recorded during the focal observations, involving eight of 21 family groups (38%) in 25 group-years (32%). Of 252 genotyped offspring, 49 were sired by extrapair males (19%), distributed in 23 of 71 broods (32%). Twenty-three extrapair offspring (9%) were sired by within-group auxiliary males, and 26 (10%) were sired by extragroup males. Among the 25 broods for which I had corresponding focal observations, there was a higher proportion of extrapair young when EPC attempts had been observed in the early breeding season of that group-year (GLM, 1.2 ± 0.3 percent, $X^2_1 = 18.7$, $P < 0.001$; Figure 4.1), although the males that actually gained EPP often differed than the males observed attempting EPCs (Table 4.1). Success of prospective extrapair males did not vary with their relatedness to the female breeder (GLMM, 0.6 ± 13.5, t (5) = 0.4, $P = 0.97$), and prospective sires were not more successful when they were less related to the female than she was to her social mate (GLMM, -1.1 ± 2.1, t (5) = -0.5, $P = 0.6$).

Success of individual males attempting EPCs and their kinship to male and female breeders are summarized in Table 4.1. In Groups 1 and 2, auxiliary males were observed repeatedly attempting EPCs with the resisting females, and they successfully attained paternity in the brood. In Group 3, three auxiliary males repeatedly attempted to mount the resisting female simultaneously, but only one of these auxiliaries attained paternity. In Group 4, an EPC attempt was observed by an auxiliary that the female did not appear to resist, but this auxiliary did not achieve paternity; another auxiliary did achieve paternity within that brood, however, even though he was never observed attempting EPCs. In Group 5, repeated EPC attempts were made by a within-group auxiliary male with the resisting female, but EPP was acquired only by extragroup male(s). Conversely, in Group 6, an EPC attempt was observed by an extragroup male on a resisting female, but EPP was instead acquired by a within-group auxiliary. In

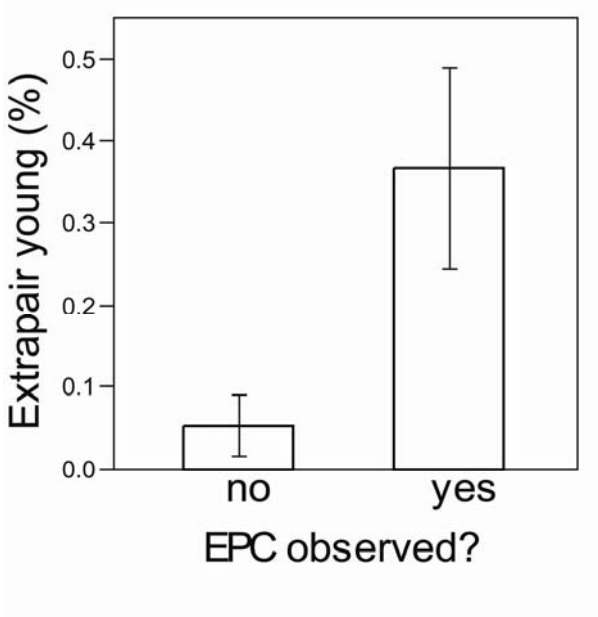

Figure 4.1. Mean proportion of extrapair offspring per brood (± SE) with observed EPC attempts in that group-year (n = 25 groups for which focal observations were conducted).

Group 7, EPCs were attempted by both a within-group auxiliary and an extragroup male, but there were no extrapair offspring in the brood. In group 8, a single extragroup male was observed attempting an EPC with an incubating female, and there were offspring sired by extragroup male(s) in her brood. In Group 9, six extragroup males attempted to simultaneously mount an incubating female, but there were no extrapair young in her brood.

Discussion

In this population, the proportion of extrapair young in a given brood was higher when EPC attempts were observed in the early breeding season of the associated group-year. In the 25 group-years for which I had conducted focal observations in the early breeding season, the proportion of group-years in which EPC attempts were observed

(32%) mirrored the overall proportion of broods in the population that contained extrapair young (32%). However, observed EPC attempts did not always predict EPP in a given brood, and failure to observe EPCs did not always predict monogamy: in two of the 25 focal group-years, there were no extrapair offspring in broods for which EPC attempts were observed, and in two other group-years, there were extrapair young in focal broods for which no EPCs were observed.

One key assumption of many studies of the evolution of EPC is that patterns of genetic paternity reflect patterns of copulatory behavior for a given female (Arnqvist and Kirkpatrick 2005, Griffith 2007), even though the relationship between observed EPCs and the overall level of EPP within populations and across species appears generally weak and nonlinear (Dunn and Lifjeld 1994, Birkhead and Moller 1995). In American Crows, the frequency of observed, attempted EPCs did indeed appear higher among females with a higher proportion of extrapair young in their broods, although the relationship between observed EPC attempts and EPP was far from a simple one. Individual males that I observed attempting EPCs were not necessarily those that attained paternity in a given brood, suggesting that a female involved in one observed EPC attempt was likely to be involved in other EPC attempts with other males in a given year.

Another frequent assumption in the literature is that it is the female that generally seeks EPCs (Westneat and Stewart 2003, Griffith 2007), and therefore females with more extrapair young in their broods are more promiscuous. As discussed elsewhere (Townsend et al. 2009b), my data do not support the idea that females sought observed EPCs: out of 15 observed EPC attempts, only one was not vigorously resisted by the female breeder. Males, not females, initiated these EPC attempts. All observed EPC attempts occurred on the females' territories. I did not observe focal females foraying into neighboring territories to solicit EPCs, although

without radio tracking data, I cannot rule out the possibility that such female forays sometimes occurred (e.g., Neudorf et al. 1997, Double and Cockburn 2000). It is also possible that EPCs solicited by females were particularly covert and therefore unlikely to be observed.

Although "forced copulations" might be uncommon among passerines (reviewed in Gowaty and Buschhaus 1998, Westneat and Stewart 2003), apparent forced copulation attempts have been described in two populations of American Crows (Kilham 1984; this study). Similar observations of resisted copulation attempts involving incubating females have been documented for two other corvids, the Northwestern Crow (*Corvus caurinus*; Verbeek and Butler 1999) and the Rook (*Corvus frugilegus*; Roskaft 1983); in the Rook, as in the Ithaca population of American Crows, these attempts sometimes involved multiple males simultaneously.

Even if females unwillingly accept copulations in order to reduce the costs of harassment, they might exercise cryptic post-copulatory choice over the success of the sperm, rejecting these undesired males (Gowaty and Buschhaus 1998). If female crows could exercise cryptic mate choice, we might expect that they would reject sperm from close relatives (particularly their sons), given the severe disease-mediated survival costs of close inbreeding in this population (Townsend et al. 2009a). Relatedness to the female breeder did not influence the success of prospective extrapair sires, however, and prospective extrapair sires were not more likely to gain paternity with a given female when they were less related to her than she was to her social mate. Three of the extrapair males that gained paternity in this study were adult sons of the female breeder (Townsend et al. 2009a; this study), suggesting that post-copulatory mate choice for the most genetically compatible mates, if it occurs in crows, might be imperfect. Another way in which females might bias the outcome of EPC attempts is by accepting EPCs from a relatively small proportion of prospective

extrapair males, who are disproportionately successful at siring offspring (Birkhead and Moller 1995, Westneat and Stewart 2003). My observations, though limited, did not support this idea. Only one of the observed EPC attempts in this study appeared to be passively accepted by the female, but this male gained no paternity in her brood.

Although the patterns of EPP with EPC suggested that certain females might have been involved in EPCs with multiple males in certain group-years, it is unclear whether this resulted from a characteristic of a given female breeder, male breeder, auxiliary male(s), or neighboring male(s). Certain females, such as those that are paired to a low-quality male, might have been more likely to accept EPCs (Jennions and Petrie 2000, Kempenaers 2007, Mays et al. 2008). Certain males might have been less able to adequately guard their females if they were in poor condition, if a large number of adult male auxiliaries competed with them for paternity, and/or if they were continually harassed by one or more extrapair males from outside of the group. Future work will examine whether the characteristics of breeders and auxiliary birds predicted patterns of EPP among broods in this population of American Crows.

REFERENCES

Akcay, E., and J. Roughgarden. 2007. Extra-pair paternity in birds: review of the genetic benefits. Evolutionary Ecology Research 9:855-868.

Arnqvist, G., and M. Kirkpatrick. 2005. The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. American Naturalist 165:S26-S37.

Arnqvist, G., and M. Kirkpatrick. 2007. The evolution of infidelity in socially monogamous passerines: a response to Griffith. American Naturalist 169:282-283.

Birkhead, T. R., and A. P. Moller. 1992. Sperm competition in birds: evolutionary causes and consequences. Academic Press, London, San Diego.

Birkhead, T. R., and A. P. Moller. 1995. Extra-pair copulation and extra-pair paternity in birds. Animal Behaviour 49:843-848.

Clark, A. B., D. A. Robinson, and K. J. McGowan. 2006. Effects of West Nile virus mortality on social structure of an American Crow (*Corvus brachyrhynchos*) population in New York state. Ornithological Monographs 60:65-78.

Double, M., and A. Cockburn. 2000. Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. Proceedings of the Royal Society of London B 267:-470.

Dunn, P. O., and J. T. Lifjeld. 1994. Can extra-pair copulations be used to predict extra-pair paternity in birds? Animal Behaviour 47:983-985.

Eliassen, S., and H. Kokko. 2008. Current analyses do not resolve whether extra-pair paternity is male or female driven. Behavioral Ecology and Sociobiology 62:1795-1804.

Gowaty, P. A., and N. Buschhaus. 1998. Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy. American Zoologist 38:207-225.

Griffith, S. C., I. P. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195-2212.

Griffith, S. C. 2007. The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection. American Naturalist 169:274-281.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews 75:21-64.

Kalinowski, S. T., Taper, M.L. & Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1106.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230-241.

Kempenaers, B. 2007. Mate choice and genetic quality: A review of the heterozygosity theory, p. 189-278. Advances in the Study of Behavior, Vol 37. Academic Press, New York.

Kilham, L. 1984. Intra- and extrapair copulatory behavior in American Crows. Wilson Bulletin 96:716-717.

Mays, H. L., T. Albrecht, M. Liu, and G. E. Hill. 2008. Female choice for genetic complementarity in birds: a review. Genetica 134:147-158.

McGowan, K. J. 2001. Demographic and behavioral comparisons of suburban and rural American Crows, p. 365-381. *In* Marzluff, J.M., Bowman, R. and Donelly, D. [eds.], Avian Ecology and Conservation in an Urbanizing World. Klumer Academic Press, Norwell, MA.

Neudorf, D. L., B. J. M. Stutchbury, and W. H. Piper. 1997. Covert extraterritorial behavior of female hooded warblers. Behavioral Ecology 8:595-600.

Pedersen, M. C., P. O. Dunn, and L. A. Whittingham. 2006. Extraterritorial forays are related to a male ornamental trait in the common yellowthroat. Animal Behaviour 72:479-486.

R Development Core Team. 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Roskaft, E. 1983. Male promiscuity and female adultery by the Rook *Corvus frugilegus*. Ornis Scandinavica 14:175-179.

Schoenle, L. A., A. K. Townsend, and I. J. Lovette. 2007. Isolation and characterization of microsatellite loci in a cooperatively breeding corvid, the American crow (*Corvus brachyrhynchos*). Molecular Ecology Notes 7:46-48.

Tarr, C. L., and R. C. Fleischer. 1998. Primers for polymorphic GT microsatellites isolated from the Mariana crow, *Corvus kubaryi*. Molecular Ecology 7:253-255.

Townsend, A. K., A. B. Clark, K. J. McGowan, E. L. Buckles, A. D. Miller, and I. J. Lovette. 2009a. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proceedings of the Royal Society B. doi:10.1098/rspb.2008.1852 (published online)

Townsend, A. K., A. B. Clark, K. J. McGowan, and I. J. Lovette. 2009b. Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crows. Animal Behaviour 77: 503-512.

Verbeek, N. A. M., and R. W. Butler. 1999. Northwestern Crow (*Corvus caurinus*). *In* Poole, A. [ed.], The Birds of North America Online. Cornell Lab of Ornithology, Ithaca.

Westneat, D. F., and I. R. K. Stewart. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology Evolution and Systematics 34:365-396.

CHAPTER 5

DIRECT BENEFITS AND GENETIC COSTS OF EXTRAPAIR PATERNITY FOR FEMALE AMERICAN CROWS *(CORVUS BRACHYRHYNCHOS)*

---

*A. K. Townsend. In Press. Direct benefits and genetic costs of extrapair paternity for female American crows (*Corvus brachyrhynchos)*. The American Naturalist.

Abstract

The idea that extrapair paternity (EPP) in birds is part of a mixed reproductive strategy driven primarily by females has incited recent controversy. In cooperatively breeding American crows, we compared predictions of four female benefits hypotheses—the genetic diversity, good genes, genetic compatibility and direct benefits hypotheses—to our predictions if EPP was primarily male-driven. We found that genetically diverse broods were not more successful, extrapair young were not in better condition or have a higher survival probability, and contrary to prediction, offspring sired by within-group extrapair males were more inbred than within-pair offspring. There was evidence of direct benefits, however: provisioning rate and number of surviving offspring were higher in groups containing within-group extrapair sires. Females therefore derived no apparent benefits from some extrapair males, and both direct benefits and genetic costs from others. We suggest that males and females both influence the distribution of EPP in this system.

Introduction

Extrapair paternity (EPP) provides socially monogamous males the opportunity to increase their reproductive success by siring offspring outside of their pair bonds, usually without the cost of parental care. Although the advantages of EPP for extrapair sires are clear, most current adaptive hypotheses for EPP in birds have emphasized the benefits of EPP for females (henceforth the "female benefits" hypotheses; reviewed in Griffith et al. 2002; Akcay and Roughgarden 2007; Kempenaers 2007; Mays et al. 2008). The idea that EPP is part of mixed reproductive strategy driven by females arose because behavioral evidence suggests that females solicit extrapair fertilizations in some taxa (reviewed in Westneat and Stewart 2003), males without intromittant

organs might be unable to fertilize unwilling females (Gowaty and Buschhaus 1998), and females are generally the choosier sex (Trivers 1972).

The most frequently invoked female benefits hypotheses are the good genes, genetic compatibility, genetic diversity, and direct benefits hypotheses (reviewed in Griffith et al. 2002; Cockburn 2004). According to the good genes and genetic compatibility hypotheses, females either select extrapair sires that are of higher genetic quality than their within-pair males, or that are more compatible to themselves than they are to their within-pair males, thereby producing extrapair young (EPY) of higher quality than their within-pair young (WPY). The genetic diversity hypothesis suggests that females seek fertilizations from multiple sires in order to produce, by chance, offspring suited to a wide variety of environmental conditions. Finally, the direct benefits hypothesis suggests that females seek a resource from extrapair sires in exchange for their paternity. Most previous studies have focused on a single female benefits hypothesis, even though females might gain one benefit but not another (Bouwman et al. 2006), females might gain multiple benefits from a single sire (Fossoy et al. 2008), or females might gain different benefits from different extrapair sires (Rubenstein 2007).

Recently, a number of models, meta-analyses and reviews have questioned whether EPP is driven primarily by the interests of male or female birds. In their review, Westneat and Stewart (2003) pointed out that extrapair copulatory behavior is rarely documented and, when observed, does not always appear to be initiated by females. Arnqvist and Kirkpatrick (2005) suggested that females accept extrapair fertilizations to alleviate harassment by extrapair males, rather than for potential benefits of EPP, because punishment by within-pair males generally outweighs genetic benefits gained by females (but see Arnqvist and Kirkpatrick 2007; Griffith 2007). Examining the same dataset, however, Eliassen and Kokko (2008) concluded that

available information is insufficient to assess whether EPP is generally male- or female-driven. Across species, meta-analyses and reviews have yielded mixed evidence for the good genes and genetic compatibility hypotheses (Akcay and Roughgarden 2007; Kempenaers 2007; Mays et al. 2008), leading some authors to suggest that these hypotheses lack general support (Akcay and Roughgarden 2007; Mays et al. 2008).

In this study, we use multiple broods from long-lived, socially monogamous American crows (*Corvus brachyrhynchos*) to simultaneously test four of the female benefits hypotheses, comparing the predictions of these hypotheses to what we would predict if EPP was primarily male-driven in this system. American crows in this population breed in cooperative family groups that include a socially monogamous pair, assisted at the nest by 0-10 auxiliaries of either sex (mean group size from 2004-2007 = 4± 0.22 birds; Townsend *in press*). Although auxiliaries are often offspring from previous broods, some auxiliaries are stepsons of the female breeder, non-descendent kin of the male breeder, or completely unrelated birds (Townsend et al. 2009b). American crows are an excellent system in which to test the female benefits hypotheses because direct benefits of EPP, which are difficult to define or measure in most taxa (Akcay and Roughgarden 2007), can be clearly defined as parental care provided by within-group extrapair sires (Cockburn 2004). Furthermore, the assumption that females are more compatible with less related males, which has been made by most tests of the genetic compatibility hypothesis (Akcay and Roughgarden 2007; but see Shields 1982; Tregenza and Wedell 2000), appears valid in this system: highly inbred offspring in this population have a relatively low survival probability (Townsend et al. 2009a). Another strength of this study system is that the frequency of observed extrapair copulation (EPC) attempts involving a given female predicts the proportion of EPY she produces (Townsend in press), suggesting that patterns of

genetic paternity can be used to infer patterns of female copulatory behavior in this population (Griffith 2007).

We examined American crow paternity in relationship to offspring condition, inbreeding and survival, as well as parental relatedness and provisioning efforts. We predicted that, if females chose extrapair sires of higher genetic quality or compatibility than their within-pair sires, then EPY would be in better condition and have a higher survival probability than WPY within individual broods. If the genetic benefits of EPP were gained from parental compatibility, then we predicted that EPY would be less inbred than WPY within these broods; similarly, we predicted that the proportion of EPY would increase with the relatedness of the social pair. If females engaged in EPC because they derive benefits from genetic diversity among their offspring, then we predicted that genetically diverse broods would generate more surviving offspring than less diverse broods, and that most or all females would engage in EPP (Bouwman et al. 2006). If females engaged in EPC to gain direct benefits from extrapair sires, then we predicted that provisioning rate would be higher in broods belonging to groups containing within-group extrapair sires, or female breeders in groups with within-group extrapair sires would themselves benefit by reducing their own provisioning efforts. Furthermore, we predicted that increased provisioning (or other forms of parental care provided by extrapair sires, such as sentinel behavior and nest defense; Wilson 2008) would lead to increased survival of all offspring in broods produced by groups containing within-group extrapair sires. The female benefits hypotheses and selected predictions are summarized in Table 5.1.

We compared these predictions of the four female benefits hypotheses to what we expected if EPP was generally male-driven in American crows. Under the male-driven scenario, females would not necessarily derive any benefits, either genetic or direct, from extragroup males. However, some predictions of the direct benefits

hypothesis (e.g., increased brood provisioning rate in groups containing within-group extrapair sires, along with increased offspring survival) were the same as what we expected if EPP was male-driven: within-group extrapair sires might invest more effort in the care of broods in which they have sired offspring, regardless of the interests of the female breeder.

Methods

Field sampling and genetic analyses

From 2004-2008, we collected genetic samples from 283 offspring belonging to 82 broods associated with 26 American crow family groups in a long-term study population in Ithaca, New York (McGowan 2001). Criteria for classifying family groups, auxiliaries, male breeders, and female breeders are described in Townsend et al. (2009b). Most auxiliaries helped to provision the incubating females, nestlings and fledglings. The 26 focal family groups were associated with 32 different pairs of social breeders, because seven breeder replacements occurred during this study. We collected DNA from all family group members of 254 of the 283 offspring, distributed in 73 broods and belonging to 26 fully genotyped pairs of social breeders. We lacked DNA from the female breeder of six social pairs, associated with 9 broods and 31 offspring. Adult DNA was extracted from blood or passively molted feathers, following Townsend et al. (2009b).

On days 20-30 after hatching, we marked live nestlings with unique combinations of metal bands, color bands and patagial tags. Each nestling was weighed and measured in tarsus, skull diameter, bill width and depth, and total length of bill. We collected blood (~150 ul) from live nestlings (n = 273) and tissue from dead nestlings (n = 10) in and under these nests. Marked offspring were monitored at least once per month until July 2008 (Townsend et al. 2009a). We were able to

Table 5.1. Summarized predictions of four hypotheses proposing that females benefit from EPP, compared with predictions if EPP was generally male-driven.

	Genetic Quality	Genetic Compatibility	Genetic diversity	Direct benefits	Male-driven
Offspring condition & survival[a]	Better for EPY	Better for EPY	No difference	No difference	No difference
Offspring inbreeding index;[a] parental similarity[b]	No difference	EPY less inbred; % EPY increases with relatedness of social pair	No difference	No difference	No difference
Brood-level success[b]	No difference	No difference	Better for broods with extragroup, extrapair sires	Better for broods with within-group extrapair sires, or the same if females reduce their own parental care	Better for broods with within-group extrapair sires
Provisioning rate[b]	No difference	No difference	No difference	Higher for broods with within-group extrapair sires, or the same if females reduce their own provisioning.	Higher for broods with within-group extrapair sires

[a]Compared among offspring within broods
[b]Compared among broods within population

monitor most offspring produced in this population throughout this study because they often remained in their natal group for a year or more, and then remained close to their natal group to breed (Clark et al. 2006).

In 2006 and 2007, we conducted one-hour nest watches on ~days 10, 15, and 20 after hatching. Some nests were only watched once or twice if they were discovered later in the nesting cycle. Hatch date was estimated by observations of the shifting behavior of female breeders when their eggs began to hatch (Caffrey 1999). We later refined nestling age estimates at the time of banding. Nest watches were rotated to conduct one watch from 05:45-06:45, one watch from 07:00-08:00, and another from 08:15-09:15 at each nest. We recorded the number of provisioning visits made by each family member, noting whether the throat of each visiting bird was distended with food and whether this food was transferred to the nestlings or incubating female. Visits without provisioning were excluded from subsequent analyses. On some occasions, only the cohort of provisioning auxiliaries could be determined.

We extracted DNA from blood samples using Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY, U.S.A.) and from feather tips using DNeasy tissue kits (Qiagen, Valencia, CA, U.S.A.). We sexed all individuals at sex linked alleles (Fridolfsson and Ellegren 1999), genotyped nestlings and their family members at ten microsatellite loci (Townsend et al. 2009b), and assessed offspring parentage using the maximum likelihood method in the program CERVUS 3.0 (Kalinowski et al. 2007). We identified probable genetic parents (within-pair and extrapair) following criteria described in Townsend et al. (2009b). Briefly, we specified female breeders as 'known parents,' and included all sampled adult males present in a given year as potential fathers. We accepted males suggested by CERVUS 3.0 as true sires when (1) they were selected as the most likely candidate at the 95% confidence level or above; (2)

they had no allelic pair mismatches; or (3) the male social breeder was selected at the 80% confidence level or more, with a single allelic pair mismatch. When the male social breeder was not the suggested sire and the confidence level for the suggested candidate fell below 95%, we denoted those offspring as having extrapair sires of unknown identity. None of the suggested sires in these latter cases were auxiliary males within the family group of the respective offspring; these extrapair sires were therefore further described as 'extragroup.' We used the program KINGROUP (Konovalov et al. 2004) to estimate relatedness coefficients between parental dyads from their microsatellite genotypes and to identify probable first- or second-order kin dyads ($R_p = 0$, $R_m = 1$, $R_p = 0$, $R_m = 0.5$; 100,000 simulations; $\alpha = 0.05$). For each offspring, we estimated internal relatedness (IR), an inbreeding index that accounts for background allele frequencies when estimating parental similarity from an offspring's microsatellite genotype (Amos et al. 2001). We have shown elsewhere that IR is an appropriate index of inbreeding in this population (Townsend et al. 2009a).

Statistical analyses

To explore the relationship between offspring body condition and parentage, we specified mass of each nestling as the response in a linear mixed effects (LME) model in JMP v. 7.0, with parentage (WPY, within-group EPY, and extragroup EPY), size, size * size, year, age, sex and all two-way interactions with parentage as fixed effects. To account for repeated observations of the same breeders over multiple years, we included social pair as a random effect. We defined nestling size as the first principal component on covariances of four structural measurements (skull, bill length, width and depth, and tarsus), which explained 96.1% of the variation. We also examined nestling size as the response, with parentage, year, age, sex and two-way interactions with parentage as fixed effects and social pair as a random effect. We

examined the relationship between offspring inbreeding index and parentage in a mixed model with IR as the response, parentage as a fixed effect and social pair as a random effect. We limited these analyses to 165 offspring belonging to social pairs that had produced both WPY and EPY in their broods. Non-significant terms were removed from final models.

Survival in relationship to parentage was examined by mark-recapture analysis in the program MARK 5.1. Capture-history matrices were constructed using resighting data from 162 individuals from the 2005-2007 cohorts for the first year after banding, divided into 10 time intervals (May-June, July, August, September, October-December, January, February, March, April, May-June). To estimate survival (Φ) and recapture (p) parameters, we first generated a set of approximating models to detect the effects of time (t) and paternity (S, grouped as WPY, within-group EPY, and extragroup EPY) on offspring Φ and p, starting with the [Φ(S*t)p(S*t)] global model. We estimated a quasi-likelihood parameter by dividing the deviance estimate from the original data by the mean of the simulated deviances from a parametric goodness of fit test (1000 bootstrap samples), adjusting the overdispersion parameter to 1.5. We compared the global model to reduced models in which we sequentially removed parentage parameters (Table 5.2). The model with the lowest quasi-Akaike Information Criterion (QAIC) score was accepted as the most parsimonious model in our set.

To test for a relationship between EPP and parental relatedness, we analyzed the proportion of extrapair offspring produced by 26 fully genotyped social pairs in a generalized linear model (GLM) in R v.2.7.2, with parental relatedness coefficients (estimated by KINGROUP) as the predictor. We specified quasibinomial errors and logit link function and weighted by the total number of offspring produced by that pair. Because males within a group are usually related to one another, females seeking

unrelated extrapair sires might prefer extragroup males. We therefore re-ran the model with proportion of offspring sired by extragroup males as the response. Parameter estimates β (±SE) are given in the logit form.

Table 5.2. Candidate set of approximating models generated to fit American crow mark-recapture data

Model	$QAIC_c$	$\Delta QAIC_c$	$QAIC_c$ weight	*Np*	Deviance
Φ(t)*p*(t)	826.6	0	0.93	17	166.8
Φ(S+t)*p*(S+t)	831.8	5.2	0.07	21	163.6
Φ(t)*p*(S*t)	848.1	21.5	0.00	33	154.1
Φ(S*t)*p*(t)	850	23.4	0.00	33	156.0
Φ(S*t)*p*(S*t)	879.5	53	0.00	51	145.1

Φ = survival; S = paternity; *p* = recapture; t = time

We examined how offspring genetic diversity and the presence of potential within-group extrapair sires affected brood-level output in two GLMMs in R v.2.7.2. We defined "genetically diverse broods" as those with at least one offspring sired by extragroup males, because males within family groups are usually relatives. We defined groups as having "potential within-group extrapair sires" if at least one auxiliary acquired some of the paternity in a given brood, or if at least one auxiliary was observed attempting to copulate with the female breeder. We defined potential sires in this way because birds might not be able to recognize their own genetic offspring (e.g., Westneat et al. 1995) and auxiliary males might therefore use attempted copulations with a given female as a way to assess their likelihood of paternity, rather than the presence of their own offspring within the brood. We examined the response variable, brood output, at two stages: the number of nestlings at

time of banding and number that survived at least six months after fledging. We specified presence of potential within-group extrapair sires (0/1), presence of extragroup EPY (0/1), year, number of adult male auxiliaries, and all two-way interactions as fixed effects, and pair as a random effect. We used a poisson distribution and log link, and present parameter estimates in log form. We excluded 12 broods sampled in 2008 from the analysis of number surviving six months after fledging, which had not yet been measured. We assumed that detectability of offspring produced by different types of sires did not vary, an assumption supported by our mark-recapture analysis (Table 5.2).

We examined provisioning rate of auxiliaries (provisioning visits / hour) in a linear mixed model with presence of potential within-group extrapair sires (0/1), year, number of nestlings, nestling age, and number of auxiliaries as fixed effects (plus two-way interactions with presence of potential within-group extrapair sires) and social pair as a random effect, in JMP v. 7.0. We could not examine individual auxiliary provisioning rates in this analysis, because we could not always distinguish among provisioning auxiliaries. We tested similar models with provisioning rates of female breeders, male breeders, and entire group as the response variables.

Results

Of 283 offspring from 83 broods belonging to 26 family groups, 234 were produced by within-pair sires (82.7%), 26 were produced by extragroup extrapair sires (9.2%) and 23 were produced by within-group extrapair sires (8.1%). Ten offspring sired by within-group extrapair sires were produced from mother-son matings. Fifteen of 29 female breeders (52%) did not have EPY in any of their broods. Among 26 fully genotyped social pairs, KINGROUP identified five pairs (19.2%) that were likely to be second-order kin. No socially monogamous pairs were likely first-order kin.

Parentage did not affect nestling mass (LME with parentage, size, size * size, year and sex as fixed effects and social pair as a random effect; $F_{2,148.4} = 0.78$, $P = 0.46$) or size (LME with parentage, age, year and sex as fixed effects and social pair as a random effect; $F_{2,144.5} = 2.03$, $P = 0.13$). Likewise, mark-recapture analysis indicated no strong paternity effects on apparent survival or recapture probability: a fully time dependent model without paternity effects was most strongly supported by the data (Δ QAIC = 5.2; Table 5.2). Although we have shown elsewhere that highly inbred offspring have a lower survival probability than relatively outbred offspring (Townsend et al. 2009a), there were too few incestuously produced EPY ($n = 10$) in this sample to analyze them separately from other EPY.

Offspring inbreeding index (IR) varied with paternity (LME with social pair as a random effect; $F_{2,161} = 6.9$, $P = 0.001$): offspring sired by within-group extrapair sires were more inbred

than WPY (Tukey's HSD, $\alpha = 0.05$; Fig. 5.1). When we removed the ten offspring produced by matings between mothers and adult auxiliary sons from the model, inbreeding index did not vary with paternity (LME with social pair as a random effect; $F_{2,149} = 0.26$, $P = 0.77$). Parental relatedness coefficient did not explain proportion of EPY for a given parental pair, either when the proportion of all extrapair young (GLM; $\beta = -0.21 \pm 1.5$, $t_{24} = -0.14$, $P = 0.89$) or the proportion of extragroup extrapair young (GLM; $\beta = -2.5 \pm 2.0$, $t_{24} = -1.2$, $P = 0.22$) were considered as the response.

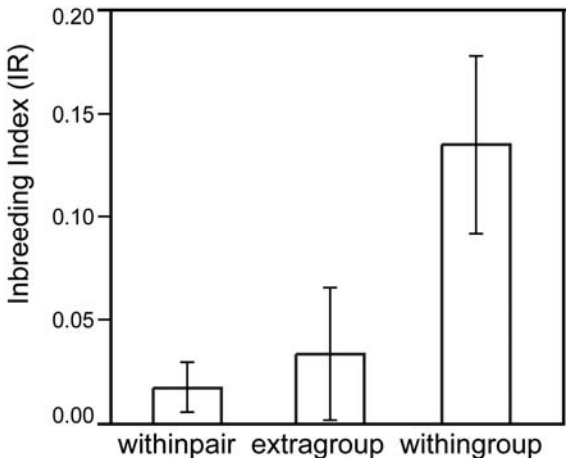

Figure 5.1. Mean inbreeding index ± SE of offspring produced by within-pair sires, extragroup extrapair sires, and within-group extrapair sires.

We conducted 99 nest watches on 30 broods from 20 family groups in 2007 and 2008. Mean provisioning rates for male and female breeders, all auxiliaries combined, and for all group members together were 2.3 ± 2.0, 0.3 ± 0.1, 3.0 ± 0.3, and 6.6 ± 3.7 visits per hour, respectively. After accounting for number of auxiliaries in the group, auxiliary and overall provisioning rates were higher at broods with potential within-group extrapair sires (Table 5.3). Although we could not quantify individual auxiliary provisioning rates in this study, the increased provisioning rate in groups with multiple potential within-group sires did appear to be driven by a markedly high

Table 5.3. Provisioning rates (visits/hour) and presence of potential within-group extrapair sires*

Response variable and fixed effects	β ± SE	df	*t*	*P*
Auxiliary provisioning rate				
Within-group extrapair sires	2.3 ± 0.8	32.6	2.8	0.008
# auxiliaries	0.6 ± 0.21	32.6	3.0	0.005
Overall provisioning rate				
Within-group extrapair sires	2.27 ± 0.8	19.9	2.8	0.01
# auxiliaries	0.7 ± 0.2	22.0	3.5	0.002
Year	-1.6 ± 0.7	91.6	-2.2	0.03
# nestlings	1.04 ± 0.27	48.8	3.9	0.004
Nestling age (days)	0.2 ± 0.05	92.7	3.9	0.002
Male provisioning rate				
Within-group extrapair sires	-0.8 ± 0.5	18.5	-1.6	0.13
Female provisioning rate				
Within-group extrapair sires	-0.13 ± 0.2	21.1	-0.9	0.37
Year	-0.5 ± 0.1	94.3	-3.5	<0.001
# nestlings	0.13 ± 0.1	51.13	2.6	0.01

*Analyzed in LMEs with social pair as a random effect.

provisioning rate by potential within-group extrapair sires (A. K. Townsend, personal observation). The provisioning rate of male and female breeders did not change when multiple potential within-group sires were present (Table 5.3), suggesting that cuckolded males did not punish their females, and that extrapair sires did not lighten breeder workload.

Broods with potential within-group extrapair sires produced more nestlings (GLMM with social pair as a random effect; β ± SE when potential within-group sires present vs. absent = 0.43 ± 0.15, t_{31} = 2.8, P = 0.001) and more fledglings (GLMM with social pair as a random effect and year as a fixed effect; β ± SE when potential within-group sires present vs. absent = 0.54 ± 0.25, t_{30} = 2.1, P = 0.04). When 13 depredated broods were included in the sample, multiple within-group sires had no significant effect on number of surviving offspring at any stage (data not shown). Presence of offspring sired by extragroup extrapair sires had no detectable effect on brood output (Table 5.4).

Table 5.4. Number of offspring produced from broods associated with within-group extrapair sires and extragroup extrapair sires*

Response variable and fixed effects	β ± SE	df	*t*	*P*
# nestlings				
Within-group extrapair sires	0.43 ± 0.15	30	2.8	0.001
Extragroup extrapair sires	0.11 ± 0.12	30	0.88	0.39
# surviving and detected to six months				
Within-group extrapair sires	0.54 ± 0.25	29	2.1	0.04
Extragroup extrapair sires	0.07 ± 0.21	29	0.3	0.74
Year[a]	0.19 ± .09	29	2.0	0.05

*Analyzed in generalized linear mixed models with social pair as a random effect

Discussion

In our population of American crows, females derived no apparent benefits from extragroup extrapair sires. Counter to the predictions of the good genes and genetic compatibility hypotheses, EPY were not in better condition, less inbred, or have a higher survival probability than WPY, and the frequency of cuckoldry did not increase with the relatedness of the social pair. Counter to the predictions of the genetic diversity hypothesis, most females did not engage in EPP, and brood output was not higher from diverse broods. From within-group extrapair sires, however, females appeared to both suffer costs and gain benefits. Ten offspring produced by within-group extrapair sires (constituting 4% of all sampled offspring) resulted from matings between mothers and their adult auxiliary sons, leading to higher mean inbreeding indices for within-group EPY than for WPY. Previously in this population, we have shown that offspring with high inbreeding indices have a lower probability of survival than relatively outbred birds (Townsend et al. 2009a). In this study, however, any reduction in survival probability of incestuously produced EPY appeared to have been outweighed at the brood level by the direct benefits provided by within-group extrapair sires. There was a higher number of surviving offspring in groups containing

multiple potential within-group sires, which might have been due, in part, to the higher auxiliary provisioning rate in these groups.

There are at least two interpretations of our results. First, females might choose to engage in EPCs for the direct benefits they gain from within-group extrapair sires, and for some unmeasured benefit gained from extragroup extrapair sires. They might, for example, choose to mate with extragroup males for fertility insurance (Griffith et al. 2002), to gain superior alleles for a trait that we did not measure (e.g., competitiveness; Kempenaers 2007) or for an additional direct benefit (e.g., territory access; Gray 1997).

The second interpretation is that EPP is primarily a male-driven strategy that is variously costly, beneficial, and neutral for female American crows. Females did not appear to derive any of our hypothesized benefits from fertilizations by extragroup extrapair sires. The direct benefits that they accrued from potential within-group extrapair sires might have been incidental to the interests of extrapair sires themselves, who had a personal fitness interest in maximizing the survival probability of their own offspring, in addition to their interest in the success of their non-descendent kin in the brood. The male-driven interpretation is supported by behavioral observations of EPCs in American crows (Kilham 1984; Townsend et al. 2009b): female crows appear to resist EPC attempts by extrapair males from both within and outside of their groups. Some of the males observed attempting to force EPCs ultimately succeed in siring offspring with these females (Townsend in press), consistent with the idea that females might sometimes accept unwanted EPCs to reduce the costs of harassment (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005). Eliassen and Kokko (2008) suggested that females should accept unwanted EPCs when the costs of resisting them exceed the costs of accepting them. In crows, accepting EPCs did not appear to lead to a reduction in male parental care, the most widely proposed cost (e.g., Eliassen and

Kokko 2008), although accepting EPCs might have entailed other costs (e.g. inbreeding depression).

This study has added to other studies suggesting that breeding females gain extra help from extrapair males in cooperatively breeding birds (Li and Brown 2002; Rubenstein 2007; but see Williams and Hale 2008). We note, however, that correlations between EPP and the direct benefits that we observed were not necessarily causal. It is possible, for example, that auxiliary males that are relatively good providers are also those that are more likely to attempt EPCs. Furthermore, even if EPP does lead to direct benefits in cooperative systems, these results might not be widely applicable to non-cooperative systems. Despite the fact that the direct benefits hypothesis is among the leading hypotheses set forth to explain the occurrence of EPP in birds, the forms that direct benefits might take are in non-cooperative species are less clear (although see Gray 1997).

In cooperatively breeding species, individuals sometimes interact with related adults of the opposite sex, and the opportunity for inbreeding is therefore relatively high (Alexander 1974). In addition to occasional mother-son incest, 19% of social pairs in this population of crows appeared to be second-order kin. Inbreeding depression has been hypothesized as a driving force behind sex-biased dispersal in many taxa (Charlesworth and Charlesworth 1987), and the regular occurrence of inbreeding in this large, open population of crows with severe inbreeding depression begs explanation (Townsend et al 2009a). In the case of the mother-son incest, the cost of lower survival probability of the most inbred individual offspring might have been outweighed, at the brood level, by increased parental efforts by the auxiliary sires. Kin selection might also play a role in the occurrence of inbreeding in this population: a female breeding with a related pair male or auxiliary son is improving the mating success of a relative, thereby increasing her own inclusive fitness (Kokko and Ots

2006), as long as some of these offspring survive. Inbreeding costs might be further defrayed if inbreeding enhances within-group cooperation (Alexander 1974) or maintains locally selected gene complexes (Shields 1982).

Most recent examinations of variation in EPP have focused solely on the idea that breeding females drive the occurrence of EPP among individuals within a population. Across species, however, support for the female benefits hypotheses has been limited and mixed (Akcay and Roughgarden 2007; Mays et al. 2008) and some authors have proposed that EPP might be primarily male-driven (Arnqvist and Kirkpatrick 2005). In this population of American crows, the patterns of parental behavior and offspring characteristics that we observed could be interpreted as consistent with either male- or female-driven EPP. We suggest that neither sex was solely responsible for driving the observed patterns of EPP in American crows. Rather, extrapair fertilizations were likely to reflect the dynamic conflicting or coinciding interests among the within-pair and extrapair males, as well as the female breeders (see Westneat and Stewart 2003). We suggest that, by considering the interests of all of these involved players, we would have a better understanding of the distribution of EPP among individuals within this population.

REFERENCES

Akcay, E., and J. Roughgarden. 2007. Extra-pair paternity in birds: review of the genetic benefits. Evolutionary Ecology Research 9:855-868.

Alexander, R. D. 1974. The evolution of social behavior. Annual Review of Ecology and Systematics 5:325-383.

Amos, W., J. W. Wilmer, K. Fullard, T. M. Burg, J. P. Croxall, D. Bloch, and T. Coulson. 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society of London B 268:2021-2027.

Arnqvist, G., and M. Kirkpatrick. 2005. The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. American Naturalist 165:S26-S37.

—. 2007. The evolution of infidelity in socially monogamous passerines: a response to Griffith. American Naturalist 169:282-283.

Bouwman, K. M., T. Burke, and J. Komdeur. 2006. How female reed buntings benefit from extra-pair mating behaviour: testing hypotheses through patterns of paternity in sequential broods. Molecular Ecology 15:2589-2600.

Caffrey, C. 1999. Feeding rates and individual contributions to feeding at nests in cooperatively breeding western American Crows. The Auk 116:836-841.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Clark, A. B., D. A. Robinson, and K. J. McGowan. 2006. Effects of West Nile virus mortality on social structure of an American Crow (*Corvus brachyrhynchos*) population in New York state. Ornithological Monographs 60:65-78.

Cockburn, A. 2004. Mating systems and sexual conflict. Pages 81-101 *in* W. D. Koenig, and J. L. Dickinson, eds. Ecology and evolution of cooperative breeding in birds. Cambridge University Press, Cambridge.

Eliassen, S., and H. Kokko. 2008. Current analyses do not resolve whether extra-pair paternity is male or female driven. Behavioral Ecology and Sociobiology 62:1795-1804.

Fossoy, F., A. Johnsen, and J. T. Lifjeld. 2008. Multiple genetic benefits of female promiscuity in a socially monogamous passerine. Evolution 62:145-156.

Fridolfsson, A. K., and H. Ellegren. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116-121.

Gowaty, P. A., and N. Buschhaus. 1998. Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy. American Zoologist 38:207–225.

Gray, E. M. 1997. Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. Animal Behaviour 53:625-639.

Griffith, S. C. 2007. The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection. American Naturalist 169:274-281.

Griffith, S. C., I. P. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195-2212.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1106.

Kempenaers, B. 2007. Mate choice and genetic quality: a review of the heterozygosity theory. Pages 189-278 *in* Advances in the study of behavior, Vol 37. Academic Press, New York.

Kilham, L. 1984. Intra- and extrapair copulatory behavior in American Crows. Wilson Bulletin 96:716-717.

Kokko, H., and I. Ots. 2006. When not to avoid inbreeding. Evolution 60:467-475.

Konovalov, D. A., C. Manning, and M. T. Henshaw. 2004. KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. Molecular Ecology Notes 4:779-782.

Li, S. H., and J. L. Brown. 2002. Reduction of maternal care: a new benefit of multiple mating? Behavioral Ecology 13:87-93.

Mays, H. L., T. Albrecht, M. Liu, and G. E. Hill. 2008. Female choice for genetic complementarity in birds: a review. Genetica 134:147-158.

McGowan, K. J. 2001. Demographic and behavioral comparisons of suburban and rural American Crows. Pages 365-381 *in* J. M. Marzluff, R. Bowman, and D.

Donelly, eds. Avian Ecology and Conservation in an Urbanizing World. Klumer Academic Press, Norwell, MA.

Rubenstein, D. R. 2007. Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. Proceedings of the Royal Society of London B 274:1895-1903.

Shields, W. M. 1982, Philopatry, Inbreeding, and the Evolution of Sex. State University of New York Press, Albany.

Townsend, A. K. in press. Extrapair copulations predict extrapair fertilizations in the American Crow. The Condor.

Townsend, A. K., A. B. Clark, K. J. McGowan, E. L. Buckles, A. D. Miller, and I. J. Lovette. 2009a. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proceedings of the Royal Society of London B 276: 2057–2064.

Townsend, A. K., A. B. Clark, K. J. McGowan, and I. J. Lovette. 2009b. Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crows. Animal Behaviour 77:503-512.

Tregenza, T., and N. Wedell. 2000. Genetic compatibility, mate choice and patterns of parentage: an invited review. Molecular Ecology 9:1013-1027.

Trivers, R. 1972. Parental investment and sexual selection. Pages 136–179 *in* B. Campbell, ed. Sexual selection and the descent of man. Heinemann, London.

Westneat, D. F., A. B. Clark, and K. C. Rambo. 1995. Within brood patterns of paternity and paternal behavior in red-winged blackbirds. Behavioral Ecology and Sociobiology 37:349-356.

Westneat, D. F., and I. R. K. Stewart. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology Evolution and Systematics 34:365-396.

Williams, D. A., and A. M. Hale. 2008. Investment in nesting activities and patterns of extra- and within-group genetic paternity in a cooperatively breeding bird. Condor 110:13-23.

Wilson, T. M. 2008. Patterns of sentinel behavior at the nest in the cooperatively breeding American crow (*Corvus brachyrhynchos*). Binghamton University, Binghamton.

CHAPTER 6

MALE INJURY PREDICTS PATERNITY LOSS IN THE COOPERATIVELY BREEDING AMERICAN CROW

Abstract

For socially monogamous pair males, injuries might lead to a higher probability of paternity loss if they cause reductions in functional fertility, mate-guarding ability, or parental care. We examined the relationship between male injuries and paternity in 94 broods, produced by 26 American Crow family groups from 2004 to 2009. Among the 116 adult birds associated with these family groups, 20 (17.2%) sustained severe wing or foot injuries over the course of this study, including 6 of 26 focal pair males (23.1%). Mean brood size of injured males was lower than of uninjured males, and injured males had a higher proportion of extrapair young in their broods. Inefficient mate-guarding by injured males did not appear to explain paternity loss: injured males maintained a similar distance to their females as uninjured males, and the rate of extrapair copulation (EPC) attempts was not higher for females paired to injured males. Extrapair young in some of the broods belonging to injured males were sired by extrapair males outside of the family group, suggesting that these females were not accepting EPCs solely to gain extra parental care. We suggest that females paired to injured males might accept EPCs to protect themselves against potential functional infertility of their mates, or that injured pair males might be at a disadvantage in sperm competition with extrapair males.

Introduction

Extrapair paternity (EPP) occurs in the majority of socially monogamous birds, but its frequency varies extensively among species, populations, and among individuals within populations (Griffith et al. 2002). Numerous phenotypic traits have been shown to correlate with the success of individual males in gaining extrapair fertilizations or protecting paternity within their own broods (Kempenaers et al. 1997). These phenotypic traits include song characteristics (Forstmeier et al. 2002), age (Dickinson

2001; Lubjuhn et al. 2007; Schmoll et al. 2007), and personality (Van Oers et al. 2008). Phenotypic variation might correlate with variation in EPP if these traits reflect genetic quality, and if, by preferentially mating with males with certain traits, females can thereby produce offspring of relatively high genetic quality (the "good genes hypothesis;" reviewed in Jennions and Petrie 2000; Griffith et al. 2002; Westneat and Stewart 2003). Recent multi-species reviews and meta-analyses of the good genes hypothesis and other genetic benefits hypotheses have shown that their support is limited, however (Akcay and Roughgarden 2007; Kempenaers 2007; Mays et al. 2008), and some authors have suggested that alternative hypotheses should be considered (Westneat and Stewart 2003; Mays et al. 2008).

There are a number of alternative hypotheses that would explain correlations between male phenotype and patterns of within- and extrapair paternity. For example, phenotypic traits might correlate with the ability of a male to fertilize females (his "functional fertility;" Sheldon 1994). Lower functional fertility (which can occur for reasons including temporary or permanent sterility, low sperm counts, and the inability to successfully transfer spermatozoa) could lead to patterns of higher reproductive success for males with certain traits (Sheldon 1994), with or without female preference for these traits. Alternatively, these traits might correlate with the ability of a given male to effectively guard his own female, to circumvent mate-guarding of other males, or to overcome female resistance to extrapair copulations. Such correlations might be expected, for example, for males that are older or in better condition (Westneat and Stewart 2003). Finally, these traits might reflect the ability of a male to provide direct resources, which a female might gain by preferentially mating with males with certain traits. This latter hypothesis, termed the direct benefits hypothesis, applies to species in which extrapair males can provide direct resources, such as access to extrapair territories (e.g., Gray 1997). In cooperatively breeding species, direct benefits can take

the form of additional parental care (e.g., offspring provisioning) provided by within-group extrapair males (Townsend et al. in press).

If variation in within-pair and extrapair fertilization success among individual males is driven solely by female preference for genetically based traits, then paternity should be unaffected by phenotypic trait variation that is not genetically based. Injuries create one source of phenotypic trait variation that is likely to be, in general, independent of genotype. Although injuries are likely to occur, to varying degrees, in all birds, the potential relationship between injuries and EPP has received little attention in the literature.

In this contribution, we analyze how paternity loss correlates with male injuries in a marked population of American crows (*Corvus brachyrhynchos*) in Ithaca, New York. American Crows in the Ithaca population are long-lived, socially monogamous but genetically polyandrous cooperative breeders (McGowan 2001; Townsend et al. 2009b). Previously, we have shown that extrapair young are produced by males outside of the family group as well as by auxiliary males within the family group (Townsend et al. 2009b). Female American crows don't appear to solicit (and in most cases, appear to resist) extrapair fertilizations (Kilham 1984; Townsend in press). Although, in some cases, extrapair offspring appear to suffer severe genetic costs (Townsend et al. 2009a; Townsend et al. in press), females might gain direct benefits from certain extrapair sires: within-group auxiliary extrapair sires appear to provision offspring at a higher rate, which might contribute to the success of the brood (Townsend et al. in press).

Using consecutive broods produced from the same family groups over a five-year period, we asked whether injured pair males suffered greater paternity loss than uninjured pair males. We examined two severe, permanent and non-lethal injuries—foot loss and injured wings—that occur regularly in this population. Loss of paternity

for injured males could result from a reduced ability of a male to guard his mate, a reduced ability of a male to care for his offspring, or a loss of functional fertility. To begin to distinguish among these possibilities, which are not mutually exclusive, we examined 1) mate-guarding behavior and rate of extrapair copulation (EPC) attempts, 2) the identity of extrapair sires (within-group or extragroup), and 3) brood size. We predicted that, if injured males were more likely to lose paternity because they were inefficient at guarding their mates, then injured males would not maintain as close a distance to their females as uninjured males, and rate of attempted EPCs would be higher with females paired to injured males. We predicted that if females paired to injured males accept EPCs in order to gain assistance with direct parental care, then extrapair males would be within-group auxiliaries. If injured males were more likely to have extrapair offspring in their broods because of depressed functional fertility, then we predicted that, even with the addition of extrapair offspring in some broods, mean brood size of injured males would be lower.

Methods

Field sampling

From 2004 to 2009, we monitored 94 broods produced by 26 American crow family groups in a long-term suburban study population in Ithaca, New York (McGowan 2001; Clark et al. 2006; Townsend et al. 2009a; Townsend et al. 2009b). Crows in this population are socially monogamous, and family groups usually contain auxiliaries of either sex, most of which help to provision the incubating females, nestlings and fledglings. Criteria and methods for classifying family groups, auxiliaries, male breeders, and female breeders are described in Townsend et al. (2009b). The 26 focal pair males in these family groups were associated with 31 different females, because five social pairings changed over the course of this study.

Focal family groups were monitored 2-7 days per week in the early breeding season (February-May) to document group membership and social role, mate guarding, within-pair and extrapair copulations, incubation, hatching and fledging. We also documented injuries of group members, which we defined as wing injuries and foot loss. Wing injuries were characterized by the sudden onset of one wing drooping permanently and asymmetrically from the body. Foot loss in this population generally appears to follow relatively minor injuries of the feet that cause slight limps, which later become infected and swollen. This condition (known as bumblefoot) is relatively common in large birds such as crows and raptors (Redig 1979). Some of these injuries appeared to result from fights in the early breeding season between established territorial birds and conspecific intruders attempting to establish overlapping territories or to join non-natal family groups (pers. obs. AKT). These fights sometimes involved wrestling on the ground, during which birds would pin their opponent with their feet, peck at their heads and beat them with their wings, and fights in the air, during which birds would cling to each other with their feet while beating their wings and falling together through tree branches. Injured birds could still perch, hop, and mount females, although often with apparent difficulty (pers. obs. AKT). Most birds injured prior to or during this study survived throughout the study's duration.

Focal observations of mate-guarding and provisioning

To quantify the rate of EPC attempts involving the individual females that were paired to our focal males, we conducted focal observations on 21 family groups from 2007 to 2009 during the nest building, egg laying and early incubation periods (ending observations by the second day of incubation). All members of focal families were tightly associated with the female breeders on their respective territories in this early breeding period. Group activities were conspicuously centered near prospective nest trees in open habitat (e.g., yards, cemeteries, and golf courses), and therefore were

easy to monitor. Observation periods on a given day began when the breeding female of a group was first located. Observation periods ended either after a predetermined period of time, or sooner, if the female of the group (generally closely accompanied by her pair male and other group members) disappeared from sight, either behind a structure on her territory or in aerial chases of predators or conspecific intruders. We recorded all within-pair and extrapair copulation attempts during one to four focal observations per family group (15–130 min per focal observation, depending on how long a family group could be followed on a given day).

Following Townsend et al. (2009b), we estimated the distance of the social pair male from his mate at 10 minute intervals within these focal observations. When he flew out of sight, which was rare, we conservatively estimated his distance from the female breeder as the furthest distance we could see in that habitat. In some intervals we were uncertain of his location relative to the female breeder. We did not include these intervals in our analyses.

Genetic sampling and analyses

Of the 94 broods that we monitored, 86 produced offspring that hatched and survived at least 20-30 days after hatching. We collected genetic samples from 295 offspring in these 86 broods, marking live nestlings with unique combinations of metal bands, color bands and patagial tags. We collected blood (~150 ul) from the brachial vein of live nestlings (n = 285), and tissue samples from carcasses of dead nestlings found in and under these nests (n = 10). We collected DNA from all family group members of 270 of the 295 offspring, distributed in 79 broods and belonging to 26 fully genotyped pairs of social breeders. We lacked DNA from five female breeders, associated with 7 broods and 25 offspring. Genetic samples were collected from adults in the form of blood or passively molted feathers, following Townsend et al. (2009b).

DNA was extracted from blood samples using Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY, U.S.A.) and from feather tips using DNeasy tissue kits (Qiagen, Valencia, CA, U.S.A.). We sexed all individuals at diagnostic sex linked alleles (Fridolfsson and Ellegren 1999). We genotyped nestlings and their family members at ten microsatellite loci (Tarr and Fleischer 1998; Schoenle et al. 2007; Townsend et al. 2009b), and assessed offspring parentage using the maximum likelihood method in the program CERVUS 3.0 (Kalinowski 2007), identifying probable genetic parents (within-pair and extrapair) following criteria described in Townsend et al. (2009b).

Statistical analyses

To estimate the effects of pair male injury on paternity, we examined the proportion of offspring produced by extrapair males in a given brood, weighted by the total number of offspring in the brood, in a generalized linear mixed model (GLMM, function glmmPQL in library MASS) with pair male injury (0/1) as a fixed effect, specifying binomial errors and logit link function, in R v.2.7.2 (R Development Core Team, 2008). Because pair males sometimes lose paternity to adult auxiliary males within the group, we also specified presence of adult male auxiliaries and their interaction with pair male injury as fixed effects in this analysis. To account for repeated observations of the same individuals over multiple years, we included pair male identity as a random effect, and we included year as a fixed effect to account for variation among years. Parameter estimates β ($\pm$SE) are given on the logit scale. Non-significant terms were removed from all final models.

To assess whether injury affected the mate-guarding behavior of pair males, we examined the log-transformed mean distance maintained between pair males and their females as the response in a linear mixed-effects model (LME, R function lme in

library nlme) with pair male injury status (0/1) as a fixed effect and pair male identity as a random effect. Mean distances were normally distributed after log transformation, and parameter estimates are given in the log form. Elsewhere we have shown that the frequency of observed EPC attempts with a given female predict the proportion of EPY in her brood (Townsend in press). We therefore also examined the occurrence of observed EPC attempts (yes/no) in a GLMM, with pair male injuries, presence of adult male auxiliaries, number of observation minutes, and the interaction between number of adult male auxiliaries with pair male injuries as fixed effects, and pair male identity as a random effect. We specified binomial errors and logit link function, and present parameter estimates in the logit form.

We examined the effect of pair male injury on brood size (number of nestlings—live or dead—at days 20-30 after hatching) in a GLMM, specifying poisson errors and log link function. We specified pair male injury and year as fixed effects and pair male identity as a random effect. We included in this model eight broods belonging to our 26 focal males from which no nestlings were genotyped, either because all eggs in the nests failed to hatch (n = 2) or because the nests failed prior to day 20-30 after hatching for unknown reasons (n = 6).

Results

Mean (± SE) group size of focal groups in the early breeding season (February-May) was 4.2 ± 0.2 birds (range = 2-11 birds). There were 159 different individual birds that cooperated in raising the broods in our sample, including 116 sexually mature adult birds (see Townsend et al. 2009b for details about assessment of sexual maturity) and 43 yearlings that did not reach maturity, either because they were sexually immature when study ended or because they died or dispersed before reaching sexual maturity. Only adult birds showed signs of injury. Among the 116 adults, 20 birds (17.2%) were

Table 6.1. Injuries of adult focal birds in this study

	focal birds	foot injuries	wing injuries
Males	65	14 (21.5%)	1 (1.5%)
Females	49	2 (4.1%)	3 (6.1%)

injured over the course of the study (Table 6.1), six of which were among the 26 socially monogamous pair males (23.1%). These pair males were injured in 15 of 94 group-years.

Mean brood size at time of banding in the 86 genotyped broods was 3.4 ± 0.14 nestlings (range = 1-6 nestlings). Of 295 offspring from these broods, 238 were produced by within-pair sires (80.7%), 28 were produced by extragroup extrapair sires (9.5%) and 29 were produced by within-group extrapair sires (9.8%). The occurrence and identity of extrapair offspring for injured and uninjured males are shown in Table 6.2.

Table 6. 2. Number of broods produced by injured and uninjured males containing offspring sired by within-group auxiliary males or extragroup males

Male status	broods	containing within-group EPY	containing extragroup EPY
Injured	11	3 (27%)	3 (27%)
Uninjured	75	9 (12%)	13 (17%)

The mean proportion of extrapair offspring (± SE) was 42.9 ± 13% for injured pair males and 13.7 ± 3% for uninjured pair males (Fig. 6.1). The proportion of EPY in broods was higher when the pair male was injured (GLMM with pair male injury and presence of adult male auxiliaries in the group as fixed effects and pair male as a random effect: β ± SE when pair males were injured vs. uninjured = 1.9 ± 0.8, $t(58) = 3.10$, $p = 0.003$). Examining the effect of foot injuries alone (i.e., excluding broods from the one pair male with a wing injury) yielded similar results (data not shown). Mean brood size at days 20-30 after hatching was 2.3 ± 0.5 offspring for injured males and 3.3 ± 0.2 for uninjured males. Even with the presence of extrapair young in

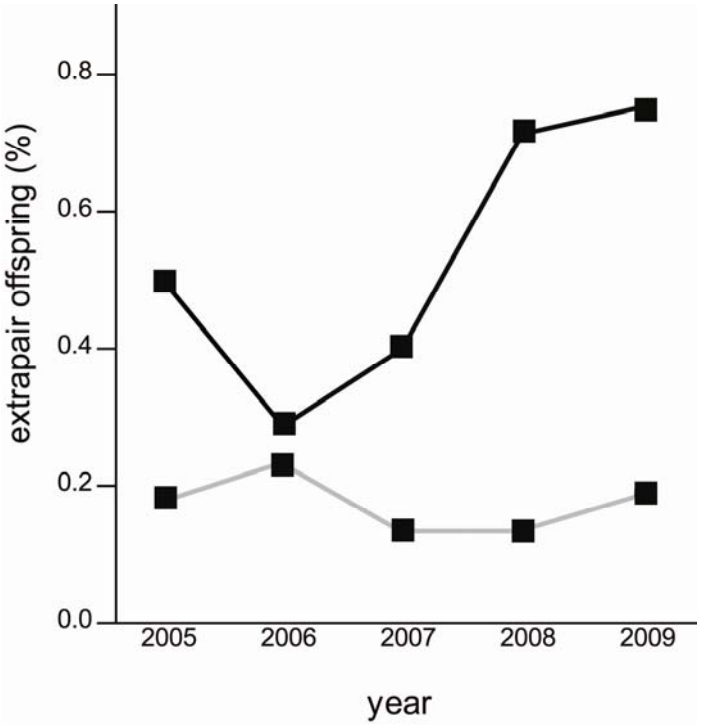

Figure 6.1. The proportion of extrapair offspring produced in broods belonging to injured males (black line) and uninjured males (gray line) from 2005 to 2009.

most of their broods, the mean number of offspring was lower in the broods of injured pair males (GLMM with pair male as a random effect: β ± SE when pair males were injured vs. uninjured = -0.39 ± 0.19, $t(67) = -2.1$, $p = 0.037$).

We conducted a total of 100.35 hours of focal observations between 2007 and 2009 (mean hours of focal observation per group-year ± SE = 3.2 ± 2.0). Mean distances between females and pair males were 13.7 ± 5.9m and 15.0 ± 3.1m for injured and uninjured pair males, respectively. Log (distance) between pair males and their mates did not differ when pair males were injured (LME with identity of pair male as a random effect; β ± SE when pair male injured vs. not injured = 0.43 ± 0.38, $t_9 = 1.12$, $p = 0.30$). We observed 11 EPC attempts in 69.8 hours of observation of 17 uninjured males in 23 group-years, and 2 EPC attempts in 30.6 hours of observation of 5 injured males in eight group-years. The likelihood of observing an EPC attempt was not higher in groups with injured pair-males (GLMM with identity of pair male as a random effect; β ± SE when pair male injured vs. not injured = -0.06 ± 0.98, $t_9 = -0.06$, $p = 0.95$).

Discussion

Among adult birds in this population of American crows, 17.2% sustained severe wing or foot injuries over the course of this study. These injuries appeared to have a reproductive cost for socially monogamous pair males: injured pair males had a higher proportion of extrapair young in their broods than uninjured males. A number of current hypotheses suggest that variation in paternity loss among individual males arises from variation in their genetic characteristics (the "genetic benefits" hypotheses, including the genetic compatibility, diversity, and quality hypotheses; reviewed in Griffith et al. 2002). Our results suggest that at least part of the variation in paternity loss might be due to phenotypic characteristics that are not genetically based. There are at least three potential explanations for the correlation between injury and paternity loss. First, females might be more likely to accept EPCs when their pair males are injured for direct benefits they might receive from the extrapair sires (the direct

benefits hypothesis); second, injured males might be less capable of successfully guarding their mates against EPC attempts (the constrained mate-guarding hypothesis); and third, injured males might have a lower functional fertility than uninjured males (the functional fertility hypothesis).

The direct benefits hypothesis for EPP suggests that females seek or accept fertilizations from extrapair males in exchange for some direct resource (usually defined as other than sperm) that the extrapair male can provide for her or her offspring. We have shown elsewhere that within-group extrapair males do appear to provide some direct provisioning benefits in this population (Townsend et al. in press). Such direct benefits of EPP might have greater value for females with injured pair males that are unable to provide sufficient parental care. If females paired to injured males generally accepted EPCs for direct benefits, we would expect these extrapair sires to be within-group auxiliary males. Within-group auxiliary males and extragroup extrapair males sired offspring in an equal number of broods belonging to injured males, however, suggesting that direct benefits cannot fully explain the loss of paternity for injured pair males.

Under the constrained mate-guarding hypothesis, we suggested that injured pair males might be less capable of preventing prospective extrapair sires from copulating with their females, or their females might be targeted for ECP attempts by extrapair males. If true, then we expected that injured pair males would not be capable of maintaining as close a distance to their females during the mate-guarding period, and that the rate at which extrapair males attempted to copulate with a given female would be higher when she was paired to an injured male. Injured pair males maintained a similar distance to their females as uninjured males, and observed EPC rate was not higher for females paired with injured males, however, suggesting that

inattentive mate-guarding does not fully explain the loss of paternity for injured pair males.

By the functional fertility hypothesis, phenotypic variation (in this case, injury) might correlate with a male's ability to fertilize a female (Sheldon 1994). Injured pair males were, in general, capable of fertilizing females: some were observed mounting females (pers. obs. AKT), and most successfully fertilized at least some offspring in their broods. It is possible, however, that injured males were less functionally fertile than uninjured males. It has been shown in humans that injured and stressed males have lower sperm counts than uninjured males (Smith 1984), which might also be true for injured birds. Furthermore, although several injured males were observed to mount females, they appeared to struggle for balance (pers. obs. AKT), which might have led to inefficient transfer of sperm to the cloaca. Reduced sperm counts or sperm transfer could lead to paternity loss for two reasons. First, even if EPC attempts on females with injured pair males were not more frequent, each EPC attempt might have been more likely to succeed because of reduced competition with the pair male's sperm (Birkhead and Biggins 1987). Second, females paired to injured males might have been more likely to accept copulations from extrapair sires (both within and outside of the group) as fertility insurance. In support of the functional fertility hypothesis, the number of offspring in broods of injured males was lower than in broods of non-injured males, even with the addition of extrapair offspring in most of these broods. We note, however, that the small brood size at day 20-30 after hatching could have been caused by factors other than lower fertility of injured males, such as reduction, full depredation or partial depredation of broods, all of which occur in this population (unpublished data). Other measures of the fertility of injured vs. uninjured males (e.g., determining the total number of spermatozoa reaching the ovum; Birkhead et al. 1994) would allow further evaluation of the functional fertility hypothesis.

Injuries in this population of crows sometimes appeared be incurred by adult birds in the process of establishing or defending a territory or breeding position (pers. obs. AKT). Although injuries in crows might be more common than in other birds because of their large size, aggressive fighting, and ability to sustain serious injuries and survive, aggressive conspecific interactions, sometimes involving physical fights (e.g., Jakobsson et al. 1995; Dunn et al. 2004), are known to occur in many species of territorial birds (Andrew 1961). A deeper understanding of the general relationship between territorial aggression, injuries and paternity might illuminate some of the unexplained variation in EPP rates observed across species, populations and individuals.

REFERENCES

Akcay E, Roughgarden J. 2007. Extra-pair paternity in birds: review of the genetic benefits. Evol Ecol Res 9:855-868.

Andrew RJ. 1961. The displays given by passerines in courtship and reproductive fighting: a review. Ibis 103:315–348; 549–579.

Birkhead TR, Biggins JD. 1987. Reproductive synchrony and extra-pair copulations in birds. Ethology 74:320-334.

Birkhead TR, Sheldon BC, Fletcher F. 1994. A comparative study of sperm-egg interactions in birds. J Reprod Fertil 101:353-361.

Clark AB, Robinson DA, McGowan KJ. 2006. Effects of West Nile virus mortality on social structure of an American Crow (*Corvus brachyrhynchos*) population in New York state. Ornith Monogr 60:65-78.

Dickinson JL. 2001. Extrapair copulations in western bluebirds (*Sialia mexicana*): female receptivity favors older males. Behav Ecol Sociobiol 50:423-429.

Dunn M, Copelston M, Workman L. 2004. Trade-offs and seasonal variation in territorial defence and predator evasion in the European Robin *Erithacus rubecula*. Ibis 146:77-84.

Forstmeier W, Kempenaers B, Meyer A, Leisler B. 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. Proc R Soc B 269:1479-1485.

Fridolfsson AK, Ellegren H. 1999. A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 30:116-121.

Gray EM. 1997. Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. Anim Behav 53:625-639.

Griffith SC, Owens IP, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195-2212.

Jakobsson S, Brick O, Kullberg C. 1995. Escalated fighting behavior incurs increased predation risk. Anim Behav 49:235-239.

Jennions MD, Petrie M. 2000. Why do females mate multiply? A review of the genetic benefits. Biol Rev Cambridge Philosophic Soc 75:21-64.

Kalinowski ST, Taper, M.L. & Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099-1106.

Kempenaers B, 2007. Mate choice and genetic quality: A review of the heterozygosity theory. In: Advances in the Study of Behavior, Vol 37. New York: Academic Press; 189-278.

Kempenaers B, Verheyren GR, Dhondt AA. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behav Ecol 8:481-492.

Kilham L. 1984. Intra- and extrapair copulatory behavior in American Crows. Wilson Bull 96:716-717.

Lubjuhn T, Gerken T, Brun J, Schmoll T. 2007. Yearling male great tits, *Parus major*, suffer more strongly from cuckoldry than older males. Zoology 110:387-397.

Mays HL, Albrecht T, Liu M, Hill GE. 2008. Female choice for genetic complementarity in birds: a review. Genetica 134:147-158.

McGowan KJ, 2001. Demographic and behavioral comparisons of suburban and rural American Crows. In: Marzluff JM, Bowman R, Donelly D, editors. Avian Ecology and Conservation in an Urbanizing World. Norwell, MA.: Klumer Academic Press; 365-381.

Redig PT, 1979. Infectious diseases. In: Cooper JE, Eley JT, editors. First Aid and Care of Wild Birds. Newton: David and Charles; 118-139.

Schmoll T, Mund V, Dietrich-Bischoff V, Winkel W, Lubjuhn T. 2007. Male age predicts extrapair and total fertilization success in the socially monogamous coal tit. Behav Ecol 18:1073-1081.

Schoenle LA, Townsend AK, Lovette IJ. 2007. Isolation and characterization of microsatellite loci in a cooperatively breeding corvid, the American crow (*Corvus brachyrhynchos*). Mol Ecol Notes 7:46-48.

Sheldon BC. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. Proc R Soc B 257:25-30.

Smith RL, 1984. Human sperm competition. In: Smith RL, editor) Sperm Competition and the Evolution of Animal Mating Systems. Orlando: Academic Press; 601-659.

Tarr CL, Fleischer RC. 1998. Primers for polymorphic GT microsatellites isolated from the Mariana crow, *Corvus kubaryi*. Mol Ecol 7:253-255.

Townsend AK. in press. Extrapair copulations predict extrapair fertilizations in the American Crow. Condor.

Townsend AK, Clark AB, McGowan KJ. in press. Genetic costs and direct benefits of extrapair paternity for female American Crows. Am Nat.

Townsend AK, Clark AB, McGowan KJ, Buckles EL, Miller AD, Lovette IJ. 2009a. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proc R Soc B.

Townsend AK, Clark AB, McGowan KJ, Lovette IJ. 2009b. Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crow. Anim Behav 77:503-512.

Van Oers K, Drent PJ, Dingemanse NJ, Kempenaers B. 2008. Personality is associated with extrapair paternity in great tits, *Parus major*. Anim Behav 76:555-563.

Westneat DF, Stewart IRK. 2003. Extra-pair paternity in birds: Causes, correlates, and conflict. Annu Rev Ecol Evol Syst 34:365-396.

CHAPTER 7

PROXIMATE MECHANISMS CONTRIBUTING TO DISEASE IN INBRED CROWS

Abstract

Cooperatively breeding American crows (*Corvus brachyrhynchos*) suffer a severe disease-mediated survival cost from inbreeding, but the proximate mechanisms linking inbreeding to disease are unknown. Here we examine indices of nestling body condition and innate immunocompetence in relationship to inbreeding, survival, and disease. Using an estimate of microsatellite heterozygosity that accurately predicts inbreeding in this population, we show that inbred crows were in relatively poor condition as nestlings, and that body condition in the first 2-33 days after hatching, in addition to inbreeding index, predicted disease probability in the first 34 months of life. Inbred nestlings also showed a lower innate immune response: the proportion of bacteria killed in a microbiocidal assay decreased as inbreeding index increased. Relatively poor body condition and low innate immunocompetence appear to be two mechanisms predisposing inbred crows to ultimate disease mortality.

Introduction

Inbreeding depression—the decline in fitness of inbred progeny relative to outbred progeny— is hypothesized to have contributed to the evolution and maintenance of sex-biased dispersal (Charlesworth and Charlesworth 1987; Szulkin and Sheldon 2008). Even in populations in which related, opposite-sex adults do interact, which occurs in some species of cooperative breeders, kin matings often appear to be avoided (Koenig and Haydock 2004), sometimes at the cost of foregoing opportunities for reproduction altogether (Koenig et al. 1999). Although inbreeding depression is well-documented in experimental and captive populations, its occurrence, manifestation and severity is not well understood in wild populations (Keller and Waller 2002; Townsend et al. 2009a), and it could be balanced or mitigated to some extent by phenomena such as outbreeding depression (Bateson 1983), kin selection

(Kokko and Ots 2006), and purging (Crnokrak and Barrett 2002). The strength of selection against inbreeding in the wild is therefore unclear.

Elevated disease risk is one potential manifestation of inbreeding depression. If, for example, the lower genome-wide heterozygosity of relatively inbred individuals correlates with lower heterozygosity at the major histocompatibility complex (MHC)—genes that have a critical role in adaptive immunity (Parslow et al. 2001)—inbred individuals might not recognize as wide a breadth of pathogens as more heterozygous individuals (Coltman et al. 1999). This heterozygote advantage hypothesis is sometimes known as overdominance (Charlesworth and Charlesworth 1999). Pathogens might also be a part of an environment that selects against individuals expressing deleterious recessive alleles (Coltman et al. 1999). For example, if expression of deleterious recessive alleles contributes to poor overall condition, inbred individuals might suffer higher mortality after infection, as has been found for individuals in poor condition for reasons other than inbreeding (Ezenwa 2004; Bradley and Altizer 2007). The hypothesis that inbreeding depression is caused by the expression of recessive alleles is sometimes known as dominance (Charlesworth and Charlesworth 1999). Experimental studies of *Drosophila* suggest that both dominance and overdominance play a role in causing inbreeding depression, although dominance effects appear to be more important (Charlesworth and Charlesworth 1999).

Empirical data concerning the disease cost of inbreeding—and the mechanistic links between inbreeding and disease—are scant from wild populations because information on both disease and level of inbreeding are often difficult to collect (although see Whiteman et al. 2006). When deep pedigree information is lacking, researchers often use heterozygosity at small panels of molecular markers as an index for individual inbreeding coefficients (Keller and Waller 2002), although molecular

markers are unlikely to reflect genome-wide heterozygosity (and inbreeding) except in rare populations that are characterized by a high frequency and variance in the occurrence of inbreeding (Balloux et al. 2004). If marker heterozygosity and genome-wide heterozygosity are not correlated in a given system, potential links between disease and inbreeding could be obscured.

Previous work has shown that close inbreeding occurs in a large, open population of cooperatively breeding American crows (*Corvus brachyrhynchos*) in Ithaca, NY: genetic analyses of parentage, parental relatedness coefficients and pedigree information suggested that 23 percent of parental dyads are first- or second order kin (Townsend et al. 2009a; Townsend in press; Townsend et al. in press). This population is characterized by a relatively high frequency and variance in the occurrence of inbreeding, the scenario under which microsatellite and genome-wide heterozygosity are most likely to be linked. Heterozygosity–heterozygosity correlations (Balloux et al. 2004) and pedigree information confirmed that a microsatellite-based index of individual heterozygosity was an appropriate index of individual genome-wide heterozygosity (and inbreeding) in this population (Townsend et al. 2009a). Using this inbreeding index, we found evidence for severe inbreeding depression: survival probability was lower for relatively inbred birds, and birds that died with disease symptoms had higher inbreeding indices than birds with other fates (Townsend et al. 2009a).

To begin assessment of the mechanistic links between inbreeding and disease probability in this population, we here examine the relationship between innate immunocompetence, body condition, inbreeding, and disease. We used a bacterial killing assay to generate indices of innate immunity (Millet et al. 2007), hypothesizing that innate immunocompetence would vary with genome-wide heterozygosity because of a heterozygote advantage at genes important in immune defense. We predicted that

relatively inbred birds would demonstrate a weaker innate immune response to a bacterial challenge than relatively outbred birds. We used a mass by size residual to generate indices of body condition (Schulte-Hostedde et al. 2005), hypothesizing that inbreeding would be linked to a general decline in body condition, and which in turn would affect disease and survival probability. We tested the specific predictions that 1) inbred birds would have relatively low nestling condition indices, 2) birds that ultimately died with disease symptoms in the duration of this study (i.e., within the first 34 months of life) would have been in relatively poor condition as nestlings than birds that had other fates, and 3) these condition indices would predict survival and disease probabilities.

Methods

Field sampling and laboratory analyses

From 2004 to 2009, we collected genetic samples and behavioral information from 354 nestlings belonging to 105 broods associated with 42 American crow family groups in a long-term suburban study population in Ithaca, New York (McGowan 2001; Clark et al. 2006; Townsend et al. 2009b). Crows in this population are socially monogamous, and family groups usually contain auxiliaries of either sex, most of which help to provision the incubating females, nestlings and fledglings. Criteria and methods for classifying family groups, auxiliaries, male breeders, and female breeders are described in Townsend et al. (2009b). Hatch date was estimated by observations of the shifting behavior of female breeders when their eggs began to hatch, and we refined nestling age estimates at the time of banding. On days 2-33 after hatching, nestlings were individually marked with temporary bands, weighed, and measured in tarsus, bill width and depth, exposed culmen, and diameter of skull (measured from the back of the head to the proximal end of the exposed culmen). We collected blood (~150 ul) from the brachial vein of live nestlings, and tissue samples from carcasses of

dead nestlings found in and under these nests. Nestlings that survived past day 20 after hatching were marked with unique combinations of metal bands, color bands and patagial tags.

Marked offspring were systematically monitored for fate at least once per month until July 2008 following Townsend et al. (2009a). Dead crows were tested for West Nile virus (WNV) via reverse polymerase chain reaction. Birds that tested negative for WNV were necropsied with a complete external and internal examination. As described in Miller et al. (in review) and Townsend et al. (2009a), dead crows discovered between November 2006 and July 2008 were subjected to gross examination and full necropsy, followed by sampling of all major organs with fixation in 10% neutral buffered formalin. Organs were sectioned using a tissue cutting knife, embedded in paraffin, microtome sectioned at 4 or 5 μm, and stained with hematoxylin and eosin using standard histological technique. Additional sections were also prepared for histochemical and immunohistochemical staining using the same protocol. All prepared sections were mounted with non-aqueous permanent mounting medium and analyzed under light microscopy by two veterinary anatomic pathologists.

DNA was extracted from blood samples using Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY, U.S.A.) and from feather tips using DNeasy tissue kits (Qiagen, Valencia, CA, U.S.A.). We sexed all individuals at diagnostic sex linked alleles (Fridolfsson and Ellegren 1999). Following Townsend et al, (2009b), we genotyped nestlings at ten microsatellite loci. For each offspring, we estimated internal relatedness (IR), an inbreeding index that accounts for background allele frequencies when estimating parental similarity from an offspring's microsatellite genotype (Amos et al. 2001), using IRMacroN4 (http://www.zoo.cam.ac.uk/zoostaff/amos/#ComputerPrograms). We have shown

elsewhere that IR is an appropriate index of inbreeding in this population (Townsend et al. 2009a).

Microbiocidal assays were carried out on nestlings sampled in 2009 following Millet et al. (2007). In brief, we diluted 0.75uL whole blood from each nestling into sterile 1.5mL capped tubes with 97.25uL of prewarmed CO_2-independent media (#18045; Gibco-Invitrogen, CA) plus 4mM L-glutamine. We used *Esherichia coli* in this assay because vertebrates are likely to have coevolved with it, and therefore are likely to have constitutive components of their immune system that respond to it (Millet et al. 2007). The strain that we used (*E. coli* ATCC #8739; American Tissue and Cell Culture, VA) was most susceptible to killing across a suite of other avian species (Millet et al. 2007). We diluted *E. coli* to a working culture in sterile PBS. Ten 10uL of the working culture (about 100 bacteria) was added to each diluted blood sample, vortexed, and incubated for 30 minutes at 41^{o}C. We vortexed the incubated samples, spread 50uL aliquots onto agar plates, inverted them, and incubated them at 37^{o}C for 24 hours. The number of bacteria in the inoculums was determined by adding the working culture to the media and L-glutamine mix (without blood) and plating 10uL. We also plated 10uL of just the media and L-glutamine mix as a negative control. The antimicrobial activity of blood was defined as the percent of the inoculums killed, calculated as 1-(viable bacteria after incubation/number inoculated).

Statistical analyses

We calculated an index of body condition for each nestling as the residual from a regression of mass against size + (size *size), defining nestling size as the first principal component on covariances of structural measurements of exposed culmen, skull, bill width and depth, and tarsus (Schulte-Hostedde et al. 2005). The first principal component explained 94.2% of the variation in these measurements. To

explore the relationship between offspring body condition and inbreeding, we specified a body condition index of each nestling as the response in a linear mixed effects model (LME; function lme in library nlme) in R v. 2.7.2, with inbreeding index, year, age, sex and all two-way interactions with inbreeding as fixed effects. To account for repeated observations of offspring produced by the same breeders over multiple years, we included social pair as a random effect. Although some of the offspring produced in these broods are sired by extrapair males, identity of sire has no directional effect on offspring body condition (Townsend et al. in press). Non-significant terms were removed from final models.

We used mark-recapture analyses in the program MARK 5.1 (http://www.phidot.org/software/mark/index.html) to examine the effects of condition and inbreeding on apparent survival. Capture-history matrices were constructed using resight data from 161 marked individuals for which we had the most consistent resight data from the 2005-2007 cohorts. Birds were followed for the first 14-34 months after hatching, depending on the year in which they hatched. Resights were collapsed into three time intervals per year (January-April, May-July, August-December). Multiple resights within intervals were treated as a single sighting. First, we generated a set of six *a priori* models to detect the effects of condition (c), inbreeding (ir), and time (t) on offspring survival (Φ) probability. Starting with the [Φ(t)*p*(t)] global model, we estimated a quasi-likelihood parameter by dividing the deviance estimate from the original data by the mean of the simulated deviances from a parametric goodness of fit test (1000 bootstrap samples), adjusting the overdispersion parameter to 1.117. We compared the global model to models in which we sequentially added the individual covariates of inbreeding indices, condition indices, and their interactions with time (Table 7.1). We did not include sex in this model set because previous analyses

Table 7.1. Candidate set of approximating models generated to fit American crow mark-recapture data. Only models with $QAIC_c$ weights > 0.05 are shown.

Model	$QAIC_c$	$\Delta QAIC_c$	$QAIC_c$ weight	*Np*	Deviance
Φ(t+ir)*p*(t)	564.6	0	0.54	18	527.3
Φ(t+ir+c)*p*(t)	566.2	1.6	0.25	19	526.7
Φ(t)*p*(t)	567.6	3.0	0.12	17	532.4
Φ(t+c)*p*(t)	568.3	3.7	0.09	18	531.0

Φ = survival; t = time; ir = inbreeding index; c = body condition index; *p* = recapture

suggested that it does not affect survival (Φ) or recapture (*p*) probabilities (Townsend et al. 2009a); likewise, we did not include time-invariant models in our model set because previous analyses suggested that there is time dependence in both recapture and survival probabilities (Townsend et al. 2009a; Townsend et al. in press). All approximating models were ranked and corrected for sample size using the quasi Akaike Information Criterion ($QAIC_c$). We assessed the importance of individual covariates by summing the normalized QAIC weights among all candidate models containing the covariate of interest. The covariate with the largest weight was considered to be the most important predictor.

Effects of nestling body condition (on days 2-33 after hatching) in addition to inbreeding on disease probability in the first 34 months of life were explored in a generalized linear model (GLM; R function glm with binomial distribution), specifying death with disease symptoms vs. all other fates (alive, traumatic deaths and unknown fates) as the response (coded as 1/0) and nestling body condition index, inbreeding index, and their interaction as predictors. We examined the relationship between bactericidal activity of the blood of nestlings and inbreeding in a generalized

linear mixed model (GLMM; R function glmmPQL in library MASS) with proportion of bacteria killed as the response (weighted by the number of bacteria in the inoculums), inbreeding coefficient, assay number, and amount of time (in hours) between sampling of blood and the assay as fixed effects, and social pair as a random effect. Parameter estimates $\beta \pm SE$ are given on the logit scale.

The relationship between immunocompetence and body condition was explored in two ways: 1) body condition index as the response in a linear mixed effects model, with inbreeding index and proportion of bacteria killed as fixed effects and social pair as a random effect; and 2) proportion of bacteria killed as the response (weighted by the number of bacteria in the inoculums) in a generalized linear mixed model with condition and inbreeding index as fixed effects and social pair as a random effect.

Results

We collected complete morphometric measurements as well as genetic sexing data from 299 offspring sampled between 2004 and 2008. A standard least squares regression suggested that condition declined with increasing inbreeding index, although there was considerable individual variation in this relationship (Fig 7.1). Likewise, in a linear mixed effects model with inbreeding index, year and sex as fixed effects and social pair as a random effect, body condition index decreased as inbreeding index increased ($\beta = -23.8 \pm 10.9$, $t(262) = -2.2$, $p = 0.03$).

In our mark-recapture analyses, we found strong evidence for an effect of inbreeding but relatively weak support for an effect of nestling body condition on apparent survival. Among the six mark-recapture approximating models in our model set, only four had appreciable support in the data ($QAIC_c$ weight > 0.05) relative to the other models (Table 7.1). A fully time dependent model with only inbreeding index as

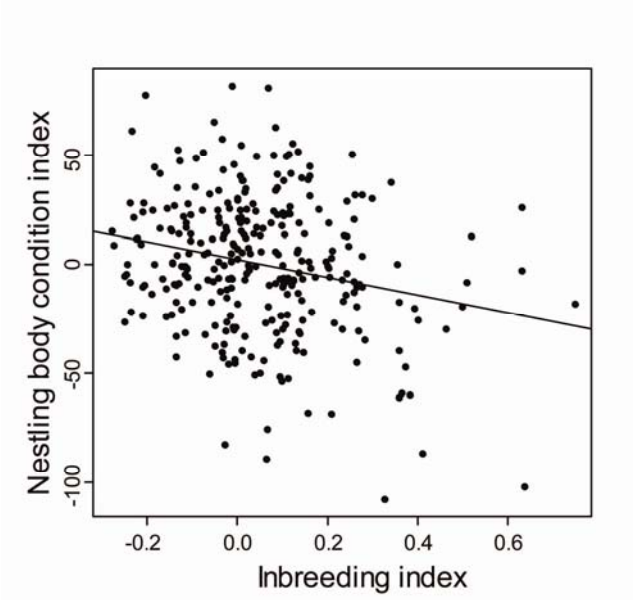

Figure 7.1. Correlation between nestling body condition index and inbreeding index ($\beta \pm SE = -42.0 \pm 10.6$; $t(297) = -4.0$, $p < 0.0001$; $r^2 = 0.05$).

a covariate was most strongly supported by the data (Δ QAIC = 1.6), and cumulative support for an inbreeding effect (0.54+0.25=0.79) was stronger than cumulative support for a condition effect (0.25+0.09=0.34).

Fates of the 299 focal nestlings from the 2004-2008 cohorts by July 2008 are described in Townsend et al. (2009a). In brief, 100 were still alive, 21 died with disease symptoms, 54 died traumatic deaths, and 124 died or disappeared of unknown causes. Lifespan for the 21 birds that died with disease symptoms ranged from 1-13 months (mean ± SE = 6.8 ± 0.9 months). Among these 299 birds, mean nestling body condition index was lower for birds that ultimately died with disease symptoms (linear model; $t(297) = -3.6$, $p = 0.0004$, Fig. 7.2A), whereas mean inbreeding index was significantly higher for birds that ultimately died with disease symptoms (linear

model; $t(297) = 4.9$, $p < 0.0001$, Fig. 7.2B). In a generalized linear model with the probability of dying with disease symptoms as a bivariate response, both inbreeding and nestling body condition were significant predictors of disease (Table 7.2).

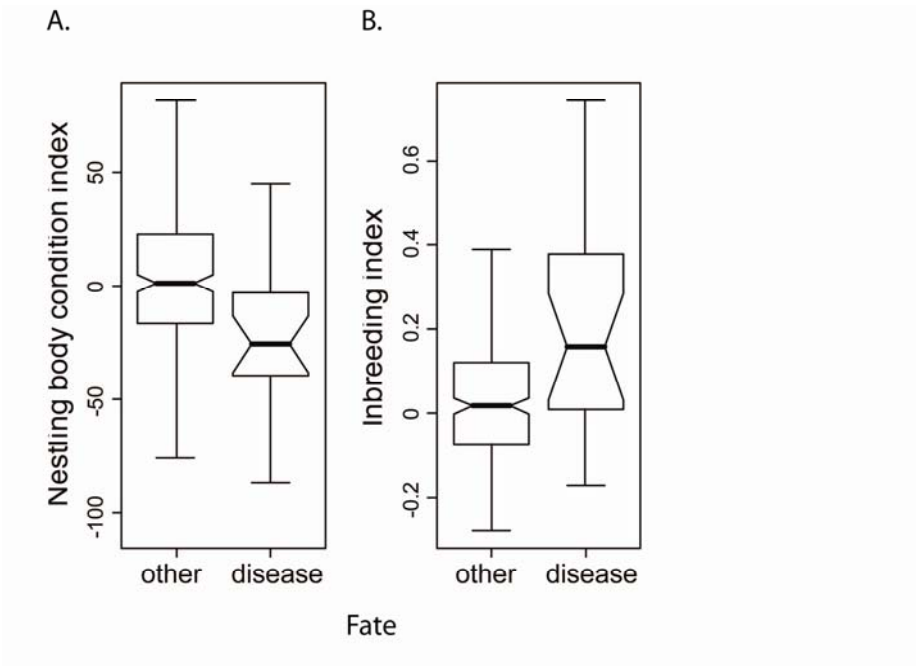

Figure 7.2. Boxplots showing the relationship between individual fates and A) nestling body condition index and B) inbreeding index. Fates were grouped as birds that died with disease symptoms ($n = 21$) and birds with other fates ($n = 180$). The horizontal line indicates the median, the bottom and top of the box indicate the 25th and 75th percentiles, respectively, and the whiskers indicate the smaller of the maximum value or 1.5 times the interquartile range. Non-overlapping notches in boxes suggest significant differences between medians.

Table 7.2. Output from a linear model showing the effects of body condition and inbreeding index on the probability of dying with disease symptoms (1 = diseased; 0 = other fates).

	β ± SE	*z*	*p*
Nestling body condition index	-0.01 ± 0.007	-2.3	0.02
Inbreeding index	4.38 ± 1.24	3.5	0.0004

A standard least squares regression suggested that the proportion of bacteria killed by whole blood declined with increasing level of inbreeding (Fig. 7.3). In a GLMM with proportion of bacteria killed as the response, inbreeding index as a fixed effect and social pair as a random effect, birds with higher inbreeding indices killed fewer bacteria (β ± SE = -1.8 ± 0.6; t(35) = -2.9, p = 0.006).

Nestling body condition and innate immune response appeared independent. Proportion of bacteria killed did not predict body condition index (LME with inbreeding index and proportion of bacteria killed as fixed effects and social pair as a random effect; -28.0 ± 15.8, t(34) = -1.8, p = 0.09), and condition index did not predict proportion of bacteria killed (GLMM with and inbreeding index as fixed effects and social pair as a random effect; -0.002 ± 0.004, t(34) = -0.6, p = 0.52).

Discussion

Previously, we have shown that inbred birds in a population of American Crows in Ithaca, NY, had a higher disease probability and lower survival probability than relatively outbred birds (Townsend et al. 2009a). The proximate mechanisms linking inbreeding to disease, however, were unknown. Here, we showed that inbred nestlings appeared to mount a weaker innate immune response than relatively outbred birds: the index. Additionally, inbred nestlings appeared to be in relatively

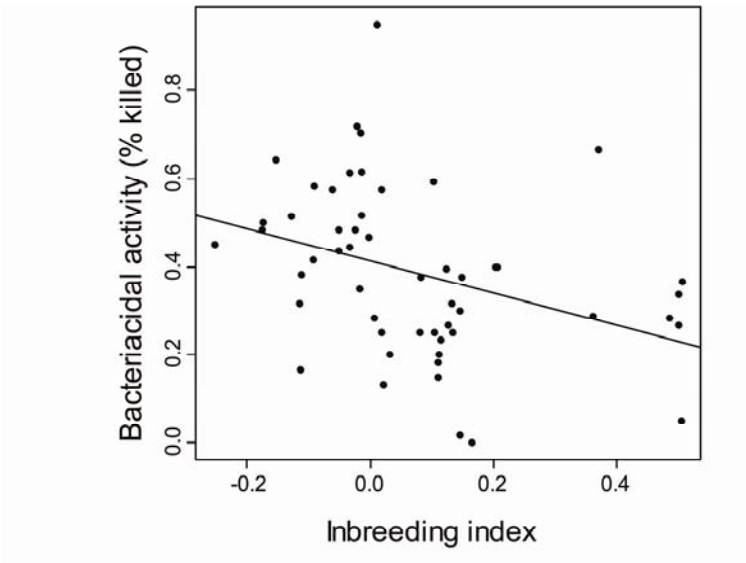

Figure 7.3. Correlation between inbreeding index and bactericidal activity against *E. coli* 8739 by diluted whole blood of crow nestlings ($\beta \pm SE = -37 \pm 0.13$; $t(51) = -2.72$, $p = 0.009$; $r^2 = 0.13$).

poor condition: body mass residuals from a regression with body size declined with inbreeding index. This body condition index appeared to have an important relationship with eventual individual fate, particularly in terms of disease. The condition of birds as nestlings was significantly lower for those that ultimately died with symptoms of disease within the first 3 years of life. Although mark-recapture analysis suggested that condition was less important than inbreeding index in predicting overall survival probability, condition had an additive effect with inbreeding in predicting disease probability: inbred birds in poor condition as nestlings had an increased likelihood of eventually dying with disease symptoms.

Our results suggest that poor nestling body condition has long-term consequences, predisposing birds in this population to eventual death by disease. Although the index of body condition was based on measurements taken when birds were only 2-33 days old, the mean age at which birds ultimately died and were

diagnosed with disease symptoms was nearly seven months after hatching. Poor condition has been linked to disease probability and mortality after infection in other species (Ezenwa 2004; Bradley and Altizer 2007), although these studies linked body condition with nutrition, not inbreeding. Likewise, in this study, there was a great deal of individual variation in the relationship between condition and inbreeding, suggesting that additional factors (genetic or extrinsic) were also affecting individual body condition. Indeed, nutrition is likely to explain some of this variation: nestling size, blood protein and calcium have been linked to habitat type in this population (McGowan 2001; Heiss et al. 2009).

Lower immunocompetence might have been another proximate mechanism contributing to the higher disease probability of inbred birds. Similar relationships between inbreeding and adaptive immunity (Reid et al. 2007) and inbreeding, ectoparasites, and innate immunity (Whiteman et al. 2006) have been described in other species, suggesting that this proximate mechanism for inbreeding depression might be widespread in wild bird populations. The bactericidal assay that we used is among the most general *in vitro* measures of the innate immune system, reflecting a combination of mechanisms that might include phagocytosis by leukocytes and microbicidal activities of humoral proteins (described in Millet et al. 2007). The exact components involved in the observed variation in immune response are therefore unknown. Additional immunocompetence assays (e.g., Matson et al. 2005; Millet et al. 2007) could illuminate the components of the immune system involved, address the potential for tradeoffs in immune response, and confirm that our results were not biased by individual variation in the production of specific antibodies (Millet et al. 2007).

Adaptive immunity can vary with body condition itself in complex ways. Although individuals in better condition might be able to mount a stronger adaptive

immune defense (Moller et al. 1998), their adaptive immune response might appear lower because they are less susceptible to infection, and they have therefore invested their energy elsewhere (Blanco et al. 2001). Innate immunity might be less dependent on body condition, and likewise, we found no suggestion that the body condition and innate immunocompetence indices were related in these nestlings.

Although a link between disease and inbreeding is a theoretical expectation (Coltman et al. 1999), evidence showing a relationship between the two in wild populations is scarce (Townsend et al. 2009a), and empirical investigations of the potential mechanisms behind this link in wild populations are even more rare (Whiteman et al. 2006; Reid et al. 2007). Experimental studies of *Drosophila* suggest that dominance is more important than overdominance in driving inbreeding depression (Charlesworth and Charlesworth 1999). Our data are consistent with the interpretation that both might play a role in linking inbreeding disease in this system if, for example, the expression of deleterious recessive alleles contributes to poor body condition, and if reductions in response to a bacterial challenge reflect lower diversity at genes important in immune defense. A deeper understanding of the contribution of overdominance and dominance could be gained, for example, by comparing MHC diversity to microsatellite diversity, immune response and disease probability in this population, and by raising relatively inbred and outbred crows under identical environments for a controlled comparison of body condition and disease susceptibility.

REFERENCES

Amos, W., J. W. Wilmer, K. Fullard, T. M. Burg, J. P. Croxall, D. Bloch, and T. Coulson. 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society B 268:2021-2027.

Balloux, F., W. Amos, and T. Coulson. 2004. Does heterozygosity estimate inbreeding in real populations? Molecular Ecology 13:3021-3031.

Bateson, P. 1983. Optimal outbreeding. Pages 257-277 *in* P. Bateson, ed. Mate Choice. Cambridge University Press, Cambridge.

Blanco, G., J. De la Puente, M. Corroto, T. Baz, and J. Colas. 2001. Condition-dependent immune defence in the Magpie: how important is ectoparasitism? Biological Journal of the Linnean Society 72:279-286.

Bradley, C. A., and S. Altizer. 2007. Urbanization and the ecology of wildlife diseases. Trends in Ecology & Evolution 22:95-102.

Charlesworth, B., and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genetical Research 74:329-340.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Clark, A. B., D. A. Robinson, and K. J. McGowan. 2006. Effects of West Nile virus mortality on social structure of an American Crow (*Corvus brachyrhynchos*) population in New York state. Ornithological Monographs 60:65-78.

Coltman, D. W., J. G. Pilkington, J. A. Smith, and J. M. Pemberton. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53:1259-1267.

Crnokrak, P., and S. C. H. Barrett. 2002. Perspective: Purging the genetic load: A review of the experimental evidence. Evolution 56:2347-2358.

Ezenwa, V. O. 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. International Journal for Parasitology 34:535-542.

Fridolfsson, A. K., and H. Ellegren. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116-121.

Heiss, R. S., A. B. Clark, and K. J. McGowan. 2009. Growth and nutritional state of American Crow nestlings vary between urban and rural habitats. Ecological Applications 19:829-839.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230-241.

Koenig, W., and J. Haydock. 2004. Incest avoidance, Pages 142-156, Ecology and Evolution of Cooperative Breeding in Birds. United Kingdom, Cambridge University Press.

Koenig, W. D., M. T. Stanback, and J. Haydock. 1999. Demographic consequences of incest avoidance in the cooperatively breeding acorn woodpecker. Animal Behaviour 57:1287-1293.

Kokko, H., and I. Ots. 2006. When not to avoid inbreeding. Evolution 60:467-475.

Matson, K. D., R. E. Ricklefs, and K. C. Klasing. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Developmental and Comparative Immunology 29:275-286.

McGowan, K. J. 2001. Demographic and behavioral comparisons of suburban and rural American Crows. Pages 365-381 *in* J. M. Marzluff, R. Bowman, and D. Donelly, eds. Avian Ecology and Conservation in an Urbanizing World. Klumer Academic Press, Norwell, MA.

Miller, A. D., K. J. McGowan, A. B. Clark, A. L. Glaser, A. K. Townsend, L. A. Patrican, E. Dobson et al. in review. Non-West Nile virus associated mortality in a population of American crows (*Corvus brachyrhynchos*): a gross and histopathologic study. Journal of Veterinary Diagnostics.

Millet, S., J. Bennett, K. A. Lee, M. Hau, and K. C. Klasing. 2007. Quantifying and comparing constitutive immunity across avian species. Developmental and Comparative Immunology 31:188-201.

Moller, A. P., P. Christe, J. Erritzoe, and A. P. Meller. 1998. Condition, disease and immune defence. Oikos 83:301-306.

Parslow, T., D. Stites, A. Terr, and J. Imboden. 2001, Medical immunology. McGraw-Hill, New York.

Reid, J. M., P. Arcese, L. F. Keller, K. H. Elliott, L. Sampson, and D. Hasselquist. 2007. Inbreeding effects on immune response in free-living song sparrows (*Melospiza melodia*). Proceedings of the Royal Society B 274:697-706.

Schulte-Hostedde, A. I., B. Zinner, J. S. Millar, and G. J. Hickling. 2005. Restitution of mass-size residuals: validating body condition indices. Ecology 86:155-163.

Szulkin, M., and B. C. Sheldon. 2008. Dispersal as a means of inbreeding avoidance in a wild bird population. Proceedings of the Royal Society B 275:703-711.

Townsend, A. K. in press. Extrapair copulations predict extrapair fertilizations in the American Crow. The Condor.

Townsend, A. K., A. B. Clark, and K. J. McGowan. in press. Genetic costs and direct benefits of extrapair paternity for female American Crows. American Naturalist.

Townsend, A. K., A. B. Clark, K. J. McGowan, E. L. Buckles, A. D. Miller, and I. J. Lovette. 2009a. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proceedings of the Royal Society B.

Townsend, A. K., A. B. Clark, K. J. McGowan, and I. J. Lovette. 2009b. Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crow. Animal Behaviour 77:503-512.

Whiteman, N. K., K. D. Matson, J. L. Bollmer, and P. G. Parker. 2006. Disease ecology in the Galapagos Hawk (*Buteo galapagoensis*): host genetic diversity, parasite load and natural antibodies. Proceedings of the Royal Society B 273:797-804.

CHAPTER 8

INBREEDING PERSISTS, WITH VARYING COSTS, IN SUBURBAN AND RURAL HABITATS OF COOPERATIVE CROWS

Abstract

Regular inbreeding and severe disease-mediated inbreeding depression has been reported in a cooperatively breeding population of American crows in suburban Ithaca, NY. Although kin selection might partly explain the evolution and maintenance of inbreeding, despite its costs, one alternative explanation is that inbreeding is an unusual occurrence for this species. We looked for evidence of spatial and temporal variation in inbreeding and inbreeding depression, using 535 crow nestlings sampled between 1998 and 2009, from the suburban population and two neighboring rural populations. Using an estimate of microsatellite heterozygosity that accurately predicts genome-wide heterozygosity, we found that mean inbreeding indices did not vary among years, even after an epidemic affected the population in 2002-2003. Likewise, inbreeding indices appeared equivalent for the suburban and rural populations. Inbreeding depression, however, appeared to vary with habitat: a nestling body condition index that has been linked to disease probability was lower for inbred birds in suburban population, but did not vary with inbreeding for rural birds. We suggest that rural immigrants dampen the response to selection against inbreeding in suburban habitats.

Introduction

The avoidance of close inbreeding is one of the few near-universals among animal species (Koenig and Haydock 2004). Therefore, populations in which close inbreeding are detected are often subject to intense investigation and speculation of its causes and consequences. Many aspects of mating behavior—such as reproductive skew (Heinsohn et al. 2000), extrapair paternity (Petrie and Kempenaers 1998), and intraspecific brood parasitism (Semel et al. 1988)—are known to vary across populations and within populations over time. Likewise, the occurrence of inbreeding

and possible inbreeding depression in a single population might not reflect its occurrence in other populations of the same species, or even its occurrence in the same population over time. Identifying such variation could greatly alter our interpretation of the causes and consequences of inbreeding in a given system.

Inbreeding has been detected at a surprising frequency in one large, open, suburban population of cooperatively breeding American crows in Ithaca, New York. In this population, opportunities for consanguineous matings are relatively high because adult birds of both sexes often remain at home to help their parents raise offspring for many years, and when they do disperse, natal dispersal distances are often short (Clark et al. 2006, Townsend et al. 2009b). Inbreeding in this population results in severe inbreeding depression: inbred birds have lower indices of body condition and immunocompetence (Townsend et al. in prep), a higher probability of dying with disease symptoms, and a lower apparent survival probability (Townsend et al. 2009a) than relatively outbred birds. The regular occurrence of inbreeding despite these costs begs explanation, and numerous factors that might lead to inbreeding and balance its costs (e.g., kin selection, direct benefits gained from extrapair sires, locally selected gene complexes, enhanced cooperation) have been proposed (Townsend et al. 2009a, Townsend et al. 2009b, Townsend et al. in press). One alternative explanation to these hypotheses is that inbreeding—or inbreeding depression—is an unusual, non-representative occurrence in this species. Inbreeding might have occurred at unusually high frequencies during the time period of these studies (2004-2008), for example, or it might be a feature unique to this particular suburban population, or to suburban populations of crows in general. Determining how universal or limited the occurrence of inbreeding and inbreeding depression is in this and other species is critical to understanding their role in the evolution of behaviors such as natal dispersal, reproductive skew, and kin recognition and avoidance.

The occurrence of inbreeding and its deleterious consequences in the Ithaca population might be linked to urbanization. Since the mid-1950s, possibly because of a reduction in human persecution, American crow densities appear to be increasing in urban areas, where pressure from predators might be lower (McGowan 2001) and access to concentrated and easily located anthropogenic food sources higher (Marzluff et al. 2001). Urban areas generally support relatively high densities of human-commensal species (Bradley and Altizer 2007); likewise, in American crow populations throughout the continental Unites States, territory size decreases as the degree of human settlement increases (Marzluff et al. 2001, McGowan 2001). McGowan (2001) showed that the density of nesting American crows was higher and number of auxiliary birds greater in this suburban Ithaca population than in neighboring rural populations. In areas of high human settlement, relatively high densities of related crows within and outside of cooperatively breeding family groups could elevate the frequency of interactions among related adults and their frequency of inbreeding.

The severity of the consequences of inbreeding, particularly in terms of disease, might also be more pronounced in urban and suburban populations than in rural populations. High population density and clumped food resources elevate conspecific contact rates of human commensal species in urban and suburban environments, favoring the transmission of parasites spread by direct contact or oral-fecal routes (Bradley and Altizer 2007). Reduced species diversity in areas of high human settlement can also lead to increased infection prevalence of vector-borne diseases, such as West Nile virus, in urban areas (Ezenwa et al. 2006, Gibbs et al. 2006, Bradley and Altizer 2007). Furthermore, suburban crows (and some other species in areas of high human settlement; Bradley and Altizer 2007; although see Schoech and Bowman 2003) show signs of malnourishment through low protein

intake (Heiss et al. 2009), which might lead to immunosuppression and higher rates of mortality after infection (Ezenwa 2004). Other features of urban and suburban living that might increase wildlife disease risk include chronic stress, a warmer microclimate, and pollutants (Bradley and Altizer 2007). If disease risk in suburban environments is much higher than in surrounding rural areas, we might expect strong selection against inbreeding (and other life-history traits) in areas with high human settlement (Shochat et al. 2006, Grimm et al. 2008).

In this contribution, we compared the occurrence of inbreeding and one measure of inbreeding depression—body condition index—between the suburban Ithaca population and two neighboring rural populations. Previous reports of inbreeding in the Ithaca population (Townsend et al. 2009a, Townsend et al. 2009b, Townsend et al. in prep, Townsend et al. in press) were based on five years of information (2004 to 2008), all of which occurred after a 2002-2003 epidemic of West Nile virus that killed many breeders and altered group structure in the population (Clark et al. 2006). The frequency of inbreeding that we reported might therefore have reflected unusual post-epidemic changes in social structure and breeding opportunities. To address this possibility, we here examine the occurrence of inbreeding using samples collected before, during and after the epidemic (1998 to 2009). Our specific goals were to determine 1) if the occurrence of inbreeding in the suburban population varied over time, particularly before and after the West Nile virus epidemic; 2) if the frequency distribution of inbreeding differed between suburban and rural populations; and 2) if inbreeding depression appeared to be less severe in rural populations.

Methods

Field sampling and laboratory analyses

We analyzed genetic samples collected from 535 nestlings, sampled from 209 broods between 1998 and 2009, belonging to 94 American crow family groups in Tompkins County, New York (McGowan 2001, Clark et al. 2006, Townsend et al. 2009b). Of these nestlings, 488 were sampled from territories in suburban Ithaca, NY (defined as "suburban/residential" and "suburban/managed" in Heiss et al. 2009), and 47 were sampled from two neighboring rural populations, approximately 40km from the Ithaca site. Rural sites were primarily undeveloped land and agricultural fields. Detailed description of the sites and environmental classification can be found in Heiss et al. (2009) and McGowan (2001). We limited our comparative analyses of nestling body condition to birds from rural and suburban/managed territories, because previous work has indicated that diet differs between suburban/residential and suburban/managed birds in complex ways (Heiss et al. 2009).

Crows in these population are socially monogamous cooperative breeders, and family groups usually contain auxiliaries of either sex, most of which help to provision the incubating females, nestlings and fledglings. Criteria and methods for classifying auxiliaries and breeders are described in Townsend et al. (2009b). Crows in this population form pair bonds that often last many years, throughout which they generally maintain the same territory. In this study, we defined “family group” by the territory in which the offspring were sampled, along with the presence of marked birds from that family from previous years (Townsend et al. 2009b). Not all offspring produced by a family group had the same genetic parents, however: ~25% of the broods in the Ithaca population contain extrapair offspring, and one or both breeders are sometimes replaced after they die or disappear (Townsend 2009, Townsend et al. 2009b, Townsend et al. in press). Breeder deaths occurred at a relatively high

frequency during the West Nile virus epidemic, which affected the Ithaca population primarily from August to October (after fledging of nestlings) in 2002 and 2003 (Clark et al. 2006). Dead crows testing positive for West Nile virus were detected at much lower levels in Ithaca after 2003 (Clark et al. 2006, Townsend et al. 2009a). In our analysis of temporal variation, we randomly selected one nestling from each brood for analysis, in order to minimize sampling of offspring produced by identical genetic parents. In other models, we accounted for non-independence among potential siblings by including family group as a random effect.

Hatch date was estimated by observations of the shifting behavior of female breeders when their eggs began to hatch, and we refined nestling age estimates at the time of banding. On days 2-33 after hatching, nestlings were individually marked with temporary bands, weighed, and measured in tarsus, diameter of skull (measured from the back of the head to the proximal end of the nares), bill width and depth, and bill from nares to tip. We collected blood (~150 ul) from the brachial vein of live nestlings, and tissue samples from carcasses of dead nestlings found in and under these nests. Nestlings that survived past day 20 after hatching were marked with unique combinations of metal bands, color bands and patagial tags.

DNA was extracted from blood samples using Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY, U.S.A.) and from feather tips using DNeasy tissue kits (Qiagen, Valencia, CA, U.S.A.). We sexed all individuals at diagnostic sex linked alleles (Fridolfsson and Ellegren 1999). Following Townsend et al, (2009b), we genotyped nestlings at ten microsatellite loci. For each offspring, we estimated individual heterozygosity as well internal relatedness, an inbreeding index that accounts for background allele frequencies when estimating parental similarity from an offspring's microsatellite genotype (Amos et al. 2001), using IRMacroN4 (http://www.zoo.cam.ac.uk/zoostaff/amos/#ComputerPrograms). Previously, we

demonstrated through heterozygosity–heterozygosity correlations and pedigree information that internal relatedness is an appropriate index of individual genome-wide heterozygosity (and inbreeding) in this population (Balloux et al. 2004, Townsend et al. 2009a). Internal relatedness was an inappropriate measure of inbreeding in the present study, however, because our available allele frequency distribution was based on allele frequencies in the Ithaca population between 2004 and 2008, which could potentially bias estimates of inbreeding in the rural populations and in the suburban population at a different time period. We therefore used individual heterozygosity in the present study, which was strongly correlated with internal relatedness (standard least squares regression; $t(544) = -123.8$, $p < 0.001$, $r^2 = 0.97$).

Statistical analyse

We examined annual variation in mean nestling heterozygosity scores, as well as differences pre-WNV (1998-2002) and post-WNV (2004-2009) using analysis of variance (ANOVA) in R v. 2.7.2. Samples were limited to a single nestling from each brood sampled in each of these years to minimize sampling of offspring produced by identical parents.

We compared heterozygosity scores of nestlings sampled from the suburban population from nestlings sampled from the two rural populations between 2005 and 2008 in a linear mixed effects model (LME; R function lme in library nlme), specifying habitat (suburban or rural) as a fixed effect and family as a random effect to account for non-independence in parentage among siblings. To explore the relationship between offspring body condition, habitat and inbreeding, we calculated an index of body condition for each nestling as the residual from a regression of mass against size + size *size, defining nestling size as the first principal component on covariances of structural measurements of skull, bill length, width and depth, and

tarsus (Schulte-Hostedde et al. 2005). The first principal component explained 94.3% of the variation in these measurements. We specified this body condition index of each nestling as the response in a linear mixed effects model, with heterozygosity score, year, age, sex, habitat and all two-way interactions with habitat and heterozygosity as fixed effects and family as a random effect. Non-significant terms were removed from the final model.

Results

Mean heterozygosity score did not differ between nestlings sampled before WNV (0.77 ± 0.01, n = 83 nestlings) and nestlings sampled after WNV (0.75 ± 0.01, n =131 nestlings; ANOVA, $F_{1,212} = 0.73$, p = 0.4). There was no apparent annual variation in heterozygosity scores (ANOVA, $F_{11,207} = 0.65$, p = 0.8; Fig. 8.1).

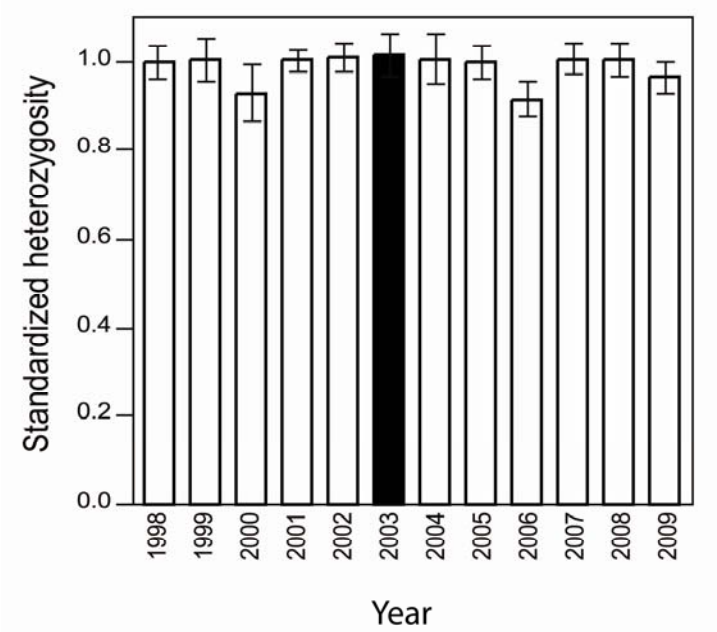

Figure 8.1. Annual variation in the distribution of nestling inbreeding indices, measured as standardized heterozygosity, from 1998-2009. The black bar indicates the West Nile virus epidemic, which affected the population primarily from August 2002 (post-breeding season) until November 2003.

Table 8.1. Linear mixed effects and habitat (residential suburban vs. rural) on nestling body condition. model* showing an interaction between heterozygosity

	β ± SE	*DF*	*T*	*P*
Heterozygosity * habitat	97.4 ± 45.1	205	2.2	0.03
Sex (male vs. female)	9.2 ± 3.6	205	2.5	0.01
Year				
2006	20.9 ± 5.9	205	3.8	<0.001
2007	23.4 ± 5.4	205	4.3	<0.001
2008	20.3 ± 5.9	205	3.4	<0.001

*Family group specified as a random effect

Among nestlings sampled between 2005-2008, mean heterozygosity score did not differ between nestlings sampled from the suburban population (0.76 ± 0.008, $n = 275$ nestlings) and nestlings sampled from the rural populations (0.75 ± 0.02, $n = 45$ nestlings; LME, $t(42) = 0.34$, $p = 0.73$). The same result was apparent when only "residential" suburban territories were compared with rural territories (i.e., when "managed" suburban territories were excluded; data not shown). There was a significant interaction between habitat and heterozygosity in predicting nestling body condition index (Table 8.1). Standard least squares regressions suggested that body condition declined as individual heterozygosity declined among suburban nestlings (Fig. 8.2A), but that body condition was independent of heterozygosity among rural nestlings (Fig. 8.2B).

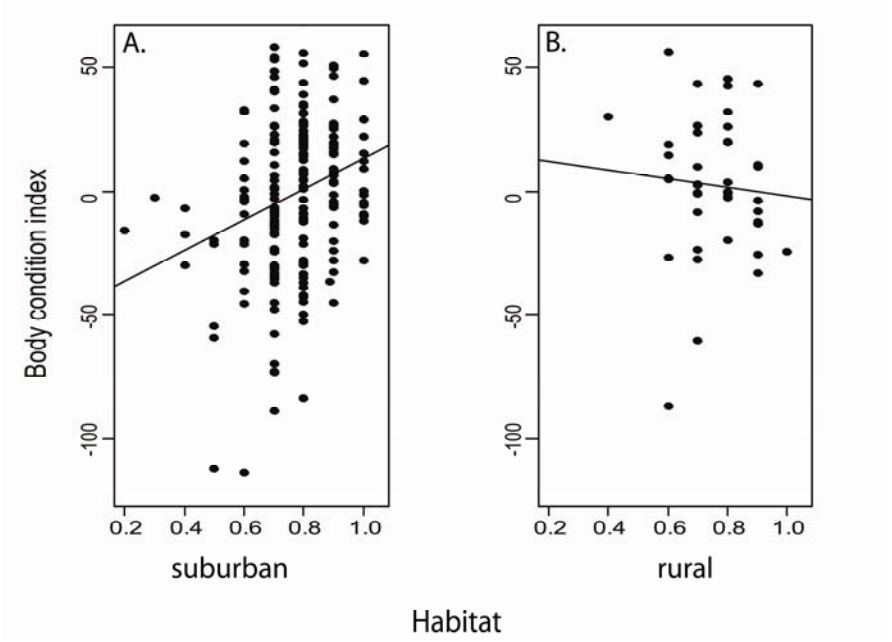

Figure 8.2. Nestling body condition index in relationship to heterozygosity, grouped by habitat (rural and suburban/residential). Nestling body condition improved with increasing heterozygosity among suburban birds ($t(198) = 3.7$, $p = 0.0002$, $r^2 = 0.07$) but not among rural birds ($t(42) = -0.5$, $p = 0.6$).

Discussion

Previous work has shown a surprisingly high level of inbreeding and severe inbreeding depression in a large, open population cooperative crows in suburban Ithaca, New York, from 2004 to 2008 (Townsend et al. 2009a). Here, we have shown that inbreeding was not a unique occurrence in suburban Ithaca over that short time period. From 1998 to 2009, a span of 11 years that encompassed a two-year West Nile virus epidemic (2002-2003; Clark et al. 2006), there were no detectable changes in mean individual heterozygosity scores of nestlings, and heterozygosity scores did not vary between the suburban Ithaca population and neighboring rural populations. The level of inbreeding depression appeared to vary across populations, however: a nestling body condition index, which has been linked to disease probability in

suburban Ithaca (Townsend et al. in prep), declined with declining heterozygosity of suburban birds, but did not vary with heterozygosity of rural birds.

In some systems, inbreeding only leads to detectable inbreeding depression under severe environmental conditions (e.g., Keller et al. 2002, Armbruster and Reed 2005, Ross-Gillespie et al. 2007, Ilmonen et al. 2008). The consequences for inbreeding for suburban crows might be greater than for rural crows because of malnourishment of suburban birds: Heiss et al. (2009) showed that the sera of suburban nestling crows contained less protein and calcium than rural birds. Protein deficiencies have been linked to a reduced immunocompetence and higher infection probabilities in other species (Ezenwa 2004), and have been proposed as one potential reason for higher disease prevalence in urban wildlife populations (Bradley and Altizer 2007; but see Schoech and Bowman 2003). Likewise, McGowan (2001) showed that nestlings produced in rural territories produced offspring that were larger and heavier than suburban nestlings, and successful rural nests produced more fledglings than suburban nests. Interestingly, however, he showed that the overall number of offspring production, as well as survival of fledglings in the first year, was higher in the suburban population than in the rural population, perhaps due in part to greater pressure by predators in the rural areas. Therefore, the costs of breeding—and inbreeding—in a suburban area in terms of body condition (and, potentially, disease; Townsend et al. in prep) might be balanced to some extent by lower costs of depredation.

If the costs of inbreeding are greater in suburban populations than in rural populations, why don't suburban crows evolve to avoid it? Although American crows have a long history of coexistence with humans (Marzluff et al. 2001), their movement into areas of high human settlement is a relatively recent phenomenon (occurring after the 1950s; McGowan 2001). Nevertheless, in other species, the microevolution of

certain traits has occurred over short time periods (Yeh 2004) and across small spatial scales (Partecke et al. 2006, Partecke and Gwinner 2007), apparently as the result of different selection regimes in human-dominated landscapes.

In crows, however, migration to and from rural areas might dampen evolutionary responses to the different selective forces in urban and suburban environments (Shochat et al. 2006). Although McGowan (2001) showed that crows tended to breed in habitats similar to the habitats in which they were born (urban/suburban or rural), habitat matching was imperfect. Also, the growing populations of crows in urban environments across the United States cannot be accounted for by urban reproductive rates, suggesting that urban populations do draw birds from other environments (Marzluff et al. 2001).

In previous contributions, we have attempted to explain the occurrence of costly inbreeding in the Ithaca population by invoking mechanisms such as increased parental efforts by the extrapair auxiliary sires (Townsend et al. in press), kin selection (Kokko and Ots 2006), enhanced within-group cooperation (Alexander 1974), or maintenance of locally selected gene complexes (Shields 1982). Nevertheless, it was difficult to explain the occurrence of extremely close inbreeding (i.e., incest), when the mortality risk for the most inbred birds was 170% higher than for the least inbred birds across the range of inbreeding index values (Townsend et al. 2009a). The persistence of inbreeding is easier to understand if the costs of inbreeding are lower in neighboring rural environments, dampening potential selection against inbreeding in areas of high human settlement. Despite the elevated inbreeding costs of suburban Ithaca, however, suburbia appears to be, overall, an attractive breeding opportunity for crows: the potential for relatively high disease risk might be outweighed by lower depredation pressure (McGowan 2001) and access to concentrated, predicable and easily accessible (albeit relatively poor quality) food sources (Marzluff et al. 2001,

Heiss et al. 2009). Neighboring environments of American crows therefore appear to offer different attractions, and crows moving across environments are subjected to different selective forces, allowing pervasive persistence of a behavior that might have severe deleterious consequences in some habitats but not others.

REFERENCES

Alexander, R. D. 1974. The evolution of social behavior. Annual Review of Ecology and Systematics **5**:325-383.

Amos, W., J. W. Wilmer, K. Fullard, T. M. Burg, J. P. Croxall, D. Bloch, and T. Coulson. 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society B **268**:2021-2027.

Armbruster, P. and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. Heredity **95**:235-242.

Balloux, F., W. Amos, and T. Coulson. 2004. Does heterozygosity estimate inbreeding in real populations? Molecular Ecology **13**:3021-3031.

Bradley, C. A. and S. Altizer. 2007. Urbanization and the ecology of wildlife diseases. Trends in Ecology & Evolution **22**:95-102.

Clark, A. B., D. A. Robinson, and K. J. McGowan. 2006. Effects of West Nile virus mortality on social structure of an American Crow (*Corvus brachyrhynchos*) population in New York state. Ornithological Monographs **60**:65-78.

Ezenwa, V. O. 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. International Journal for Parasitology **34**:535-542.

Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006. Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B **273**:109-117.

Fridolfsson, A. K. and H. Ellegren. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology **30**:116-121.

Gibbs, S. E. J., M. C. Wimberly, M. Madden, J. Masour, M. J. Yabsley, and D. E. Stallknecht. 2006. Factors affecting the geographic distribution of West Nile virus in Georgia, USA: 2002-2004. Vector-Borne and Zoonotic Diseases **6**:73-82.

Grimm, N. B., S. H. Faeth, N. E. Golubiewski, C. L. Redman, J. G. Wu, X. M. Bai, and J. M. Briggs. 2008. Global change and the ecology of cities. Science **319**:756-760.

Heinsohn, R. G., P. O. Dunn, S. Legge, and M. C. Double. 2000. Coalitions of relatives and reproductive skew in cooperatively breeding White-winged Choughs. Proceedings of the Royal Society B **267**:243-249.

Heiss, R. S., A. B. Clark, and K. J. McGowan. 2009. Growth and nutritional state of American Crow nestlings vary between urban and rural habitats. Ecological Applications **19**:829-839.

Ilmonen, P., D. J. Penn, K. Damjanovich, J. Clarke, D. Lamborn, L. Morrison, L. Ghotbi, and W. K. Potts. 2008. Experimental infection magnifies inbreeding depression in house mice. Journal of Evolutionary Biology **21**:834-841.

Keller, L. F., P. R. Grant, B. R. Grant, and K. Petren. 2002. Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. Evolution **56**:1229-1239.

Koenig, W. and J. Haydock, editors. 2004. Incest avoidance. Cambridge University Press, United Kingdom.

Kokko, H. and I. Ots. 2006. When not to avoid inbreeding. Evolution **60**:467-475.

Marzluff, J. M., K. J. McGowan, R. Donnelly, and R. L. Knight. 2001. Causes and consequences of expanding American Crow populations. Pages 365-381 *in* J. M. Marzluff, R. Bowman, and R. Donnelly, editors. Avian Ecology and Conservation in an Urbanizing World. Kluwer Academic Press, Norwell, MA.

McGowan, K. J. 2001. Demographic and behavioral comparisons of suburban and rural American Crows. Pages 365-381 *in* J. M. Marzluff, R. Bowman, and D. Donelly, editors. Avian Ecology and Conservation in an Urbanizing World. Klumer Academic Press, Norwell, MA.

Partecke, J. and E. Gwinner. 2007. Increased sedentariness in European Blackbirds following urbanization: A consequence of local adaptation? Ecology **88**:882-890.

Partecke, J., I. Schwabl, and E. Gwinner. 2006. Stress and the city: Urbanization and its effects on the stress physiology in European Blackbirds. Ecology **87**:1945-1952.

Petrie, M. and B. Kempenaers. 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends in Ecology & Evolution **13**:52-58.

Ross-Gillespie, A., M. J. O'Riain, and L. F. Keller. 2007. Viral epizootic reveals inbreeding depression in a habitually inbreeding mammal. Evolution **61**:2268-2273.

Schoech, S. J. and R. Bowman. 2003. Does differential access to protein influence differences in timing of breeding of Florida Scrub-jays (*Aphelocoma coerulescens*) in suburban and wildland habitats? The Auk **120**:1114-1127.

Schulte-Hostedde, A. I., B. Zinner, J. S. Millar, and G. J. Hickling. 2005. Restitution of mass-size residuals: validating body condition indices. Ecology **86**:155-163.

Semel, B., P. W. Sherman, and S. M. Byers. 1988. Effects of brood paratism and nest-box placement on wood duck breeding ecology. Condor **90**:920-930.

Shields, W. M. 1982. Philopatry, Inbreeding, and the Evolution of Sex State University of New York Press, Albany.

Shochat, E., P. S. Warren, S. H. Faeth, N. E. McIntyre, and D. Hope. 2006. From patterns to emerging processes in mechanistic urban ecology. Trends in Ecology & Evolution **21**:186-191.

Townsend, A. K. 2009. Extrapair copulations predict extrapair fertilizations in the American Crow. The Condor **11**:387-392.

Townsend, A. K., A. B. Clark, and K. J. McGowan. in press. Genetic costs and direct benefits of extrapair paternity for female American Crows. American Naturalist.

Townsend, A. K., A. B. Clark, K. J. McGowan, E. L. Buckles, A. D. Miller, and I. J. Lovette. 2009a. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proceedings of the Royal Society B **276**: 2057-2064.

Townsend, A. K., A. B. Clark, K. J. McGowan, and I. J. Lovette. 2009b. Reproductive partitioning and the assumptions of reproductive skew models in the cooperatively breeding American crow. Animal Behaviour **77**:503-512.

Townsend, A. K., A. B. Clark, K. J. McGowan, A. D. Miller, E. L. Buckles, and I. J. Lovette. in prep. Immunocompetence, condition and disease of inbred crows Ecology Letters.

Yeh, P. J. 2004. Rapid evolution of a sexually selected trait following population establishment in a novel habitat. Evolution **58**:166-174.